Gender Specific Effects of TRPV4 on Osteoblast-Osteoclast Coupling and Risk of Osteoporotic Fractures

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SA001

Dependence of Post Natal Osteogenic Differentiation on BMP2: Dissection of Osteogenic Lineage Commitment by Lentivirus BMP2 shRNA *ex vivo* and *in vivo*. M. V. Bais*, N. Wigner*, M. Young*, R. Toholka*, D. Graves*, E. F. Morgan*, L. C. Gerstenfeld, T. A. Einhorn. Orthopaedic Research Laboratory, Department of Orthopedic Surgery, Boston University School of Medicine, Boston, MA, 02118, Boston, MA, USA.

In order to examine if autogenous BMP-2 expression is necessary for the osteogenic differentiation of marrow stromal cells, BMP-2 expression was inhibited using lentiviral transfection of a BMP2 shRNA. Inhibition of BMP2 expression decreased the expression of markers of terminal osteoblasts (osteocalcin, Runx2, osterix, APase activity, and mineral deposition), while the expression of transcription factors (Sox 9 and Msx2) that are seen in skeletal lineage progenitors were not effected. Rescue of osteogenic differentiation by the exogenous addition of either BMP7 or BMP2 recovered the levels of expression of osterix and APase and enhanced Sox9 and Msx2. However, BMP7 did not recover the expression of Runx2 and only partially recovered mineral deposition while the exogenous addition of BMP2 recovered all of these phenotypic properties. The effect of the loss of BMP2 expression on bone formation after marrow ablation lead to 80% loss of mineralized bone formation as assayed by microCT at day seven after surgery. Assay of same set of mRNAs as examined in vitro showed transient ~2 fold increase of Sox9 at day three after surgery in the shRNA BMP2 samples while at day seven osterix, Runx2, osteocalcin and Sox9 all showed ~50 to ~80% inhibition compared to expression in the NT (scrambled) treated samples. Interestingly, Msx2 showed ~10 fold elevation expression at seven days post surgery. Immunohistological examination of the cell populations found in the meduallary space three days after surgery with antibodies for CD146 and Sox9, showed equal numbers of cells expressing these skeletal stem markers in both control and shRNA treated specimens. These results demonstrate that: 1) BMP-2 is a central morphogenetic factor for the post natal osteogenic differentiation: 2) BMP2 does not effect the initial recruitment or expansion of skeletal stem cells in vivo: 3) Both BMP2 and BMP7 can both induce osterix and this transcription factor can promote ostegenic differentiation: 4) Expression of Runx2 is dependent on BMP2: 5) Both osterix and Runx2 are needed to fully promote osteogenic development.

Figure 1 MicroCt assessment of endosteal bone formation in response to surgical marrow ablation.

Disclosures:* *M.V. Bais*, *None.
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SA002

See Friday Plenary number F002.

SA003

Clinical Application of Resorbable Polymers in Guided Bone Regeneration. W. IP*. Orthopaedics & Traumatology, The University of Hong Kong, Hong Kong, Hong Kong.

INTRODUCTION: Long segmental diaphyseal bone loss often results from high energy trauma like blast injury, osteomyelitis or wide excision of malignant conditions. Treatment of this long segmental diaphyseal defects remain a difficult clinical problem. In the literature, many authors have reported that bone loss more than 2.5 cm always require bone grafting. This is probably the critical size defect in human. Non-vascularized bone graft frequently fails if the defect is longer than 6-7 cm. 2.5 cm is probably the critical size defect in human and 7 cm is likely the critical size for non-vascularized bone graft. Various treatment methods are adopted currently to address this problem, including vascularized bone graft, distraction osteogenesis and massive allograft. However, all these methods are associated with a lot of problems.
Successful guided bone regeneration has been achieved in skull bone and jaw bone using resorbable allograft. Bone regeneration in long segmental defect and relatively small defect in tumour excision has been achieved using resorbable polylactide scaffolds.
METHODS & MATERIALS: 10 patients with bone defect of sizes up to 6 cm due to various causes including benign tumour, osteomyelitis & fractures were treated with resorbable polylactide scaffold impregnated with marrow blood which contains stromal cells. In cases with infection, antibiotics was also loaded into the scaffold and in this situation, the scaffold also served as a drug delivery device. The patients have assessed regularly with X rays and clinical symptoms.
RESULTS: Serial X ray evaluation and clinical evaluation revealed presence of bone regeneration. The limbs enjoyed satisfactory function and there was minimal donar site morbidity and major surgery can be avoided.
DISCUSSION : Selected cases are treated with guided bone regeneration which would be treated otherwise by conventional technique. Vascularized bone transfer has limited supply and involves a major operation. There is always a chance of vascular complication and there is donor site morbidity. Distraction osteogenesis has a limitation of length that can be lengthened and requires a prolonged placement of external fixation. There is a high chance of traction injury to nerve and other soft tissues. Massive allograft requires a prolonged period, in terms of decades, for complete creep substitution. There is also a high incidence of disease transmission and infection. Therefore there is a constant demand for bone substitute which can bridge long segmental defect effectively with minimal morbidity and can heal in reasonable time frame. The affected limb can be rehabilitated and bear weight for functional restoration as early as possible. These early results are promising.

Disclosures:* *W. Ip*, *None.

SA004

See Friday Plenary number F004.

SA005

CCAAT/Enhancer Binding Protein-Beta (C/EBP β) Overexpression Causes Osteopenia. S. Zanotti, A. Smerdel-Ramoya, L. Stadmeyer*, E. Canalis. Research, Saint Francis Hospital and Medical Center, Hartford, CT, USA.

C/EBPs are a family of ubiquitous transcription factors involved in cell differentiation. Six different C/EBPs are known, α, β, δ, γ, ε and ; and they can form homo- and heterodimers that bind to similar sequence motifs. C/EBPs are expressed by osteoblasts and adipocytes during differentiation *in vitro*, and C/EBP β is required for adipogenesis. However, the role of C/EBP β in osteoblastogenesis is less clear, and its function in the postnatal skeleton is not known. To study the function of C/EBP β in osteoblasts *in vivo*, we created transgenic mice expressing C/EBP β under the control of the 3.8 kilobase fragment of the human osteocalcin promoter. Two transgenic lines were established in an FVB genetic background, and four week old C/EBP β transgenic mice were compared to wild type littermates of identical sex. Both C/EBP β transgenic lines exhibited osteopenia with a 30 % decrease in trabecular bone volume over tissue volume, due to a decrease in trabecular number. There was a reduction in number of osteoblasts per tissue area, although the number of osteoblasts per bone perimeter was not changed. The phenotype was the same in both male and female transgenics. To examine the mechanism leading to osteopenia, bone marrow stromal cells and calvarial osteoblasts from C/EBP β transgenics, and ST-2 stromal cells stably transduced with a C/EBP β expression construct were studied. In accordance with the results observed *in vivo*, bone marrow stromal cells from C/EBP β transgenics showed reduced mineralization as determined by alizarin red staining, and reduced alkaline phosphatase mRNA levels. Furthermore, calvarial osteoblasts from C/EBP β transgenics and ST-2 stromal cells stably expressing C/EBP β displayed reduced alkaline phosphatase activity. In conclusion, C/EBP β overexpression *in vivo* causes osteopenia, possibly by impairing osteoblastic function.

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SA006

See Friday Plenary number F006.

SA007

Use of GFP Reporter Mice for Assessing Osteoprogenitor Cell Activity in a Critical Size Calvarial Defect. L. Wang, Y. Liu*, P. Maye, D. W. Rowe. Dept. of Reconstructive Sciences, School of Dental Medicine, University of Connecticut Health Center, Farmington, CT, USA.

A successful repair strategy of a critical size defect in bone requires the interaction of cells from the endothelial/vascular, osteoprogenitor and hematopoietic lineages and a scaffold that is permissive or proactive to this process whether the cells are derived from host or implanted donor cells. Using a variety of GFP reporter mice that reflect the cellular activities of each tissue element and a new cryohistology for non-decalcified bone that preserves GFP signals, we have developed a calvarial defect model to assess the temporal and special events of a successful repair as a baseline for understanding why some strategies may fail. In two experimental designs, one assesses the host and donor contribution to a repair, host and donor express the same promoter driving distinguishable fluorescent proteins. Thus a defect that is closed with an acellular scaffold results in Col3.6green host cells streaming into the scaffold but never making a bone matrix as judged by the lack of xylenol orange (XO) staining or gross mineral deposition that is visible by DIC optics. However when donor cell derived from neonatal calvaria carrying the Col3.6blue reporter, a DIC positive matrix is produced that contains blue osteoblasts overlying the XO staining osteoid surface. The early woven bone present at 4 weeks begins the transition to remodeled bone observed when the repair is sampled at 8 weeks. Limited intermingling of host and donor osteoblasts can be observed at the margins, but no invasion of host osteoblasts within the scaffold is detected. However host derived osteoclastic cells are abundant in the repair and are recognized as weak Col3.6+ green cells that are not associated with the XO mineral label and are TRAP positive. The second design uses a nontransgenic host that receives donor cells carrying cell specific reporters expressing distinguishable colors. When the scaffold is inoculated with fresh bone marrow from a Col3.6green donor, no bone is formed and no donor cells survive. In contrast, when the

fresh marrow is mixed with neonatal calvarial cells carrying the Col3.6blue reporter, bone is formed with blue osteoblasts overlying the XO labeled matrix. Abundant green cells are present in the 8 week sample that have the signature of osteoclastic cells. However if marrow stromal cells that are double positive for BSP-green and DMP1-red are expanded in cultures and then used to seed the scaffold, abundant bone is formed that shows active green osteoblasts on the bone surface and red osteocytes within the bone matrix. Many permutations of this approach can be applied to assess the donor/scaffold/host variables that affect the repair of a bone defect.

Disclosures: ***L. Wang****, None.*

SA008

See Friday Plenary number F008.

SA009

Bone Cell Response to Neurotransmitters and Mechanical Loading. J. H. Kwag*, B. G. Kim*, H. G. Lee*, C. H. Kim. Yonsei University, Wonju, Republic of Korea.

Mechanical loading is an important regulator of bone resorption. Also, neurotransmitters have been shown to exist in bone tissue and play a vital role in stimulating the calcium content and alkaline phosphatase level. In this study, our objective was to investigate the role of an important neurotransmitter (vasoactive intestinal peptide (VIP)) in controlling the mechanotransduction in bone cells. First, a dose study was performed. MC3T3-E1 preosteoblast cells were cultured in culture dishes with complete culture medium (CCM) for 6 days. VIP was added at either 0 (control), 10^{-9}M or 10^{-6}M on day 0 and day 3. mRNA was isolated on day 6 and real-time RT-PCR was performed. Second, the effect of VIP on mechanical loading was assessed. MC3T3-E1 cells were cultured in culture dishes and 10^{-6}M of VIP added on day 0 and day 3. Cells were subcultured onto glass slides on day 5. Oscillatory fluid flow-induced shear stress was applied on day 6 using a custom-built loading device at 1 Pa for 1 hour. mRNA was isolated immediately after end of loading and real-time RT-PCR performed. For real time-RT-PCR, expression levels of osteoprotegerin (OPG) and receptor activator for NF-kB ligand (RANKL) genes were quantified. For the dose study, RANKL decreased by 40% (10^{-9}M VIP) and 60% (10^{-6}M VIP), while OPG increased by 50% (10^{-6}M VIP) compared to control. When VIP was combined with loading, RANKL decreased by over 90% and OPG increased by approximately 70% compared to control. Results suggest that VIP may have the potential to decrease osteoclastogenesis. In addition, it appears that VIP and oscillatory fluid flow-induced shear stress may have synergistic effects in controlling the bone remodeling process.

Disclosures: ***C.H. Kim****, None.*
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SA010

See Friday Plenary number F010.

SA011

Silencing of RhoGTPases Counteract Microgravity-induced Effects on Osteoblasts. A. Guignandon*, C. Faure*, M. Linossier*, A. Rattner*, N. Laroche*, L. Vico. LBTO, INSERM U890, Saint Etienne, France.

Effects of space-related conditions on osteoblastic cells are characterized by a decrease in cell adhesion, disruption of cytoskeletal integrity leading to reduced differentiation potential. These effects can be mainly explained by deregulation of p21-RhoGTPases (p21), well-known regulators of actin-cytoskeleton. We exposed MG63 osteoblastic cells to 3 days of microgravity (µg) during Foton M3 ESA mission and compared them to ground controls (1g) kept in similar conditions except µg. Specific p21 activities are altered by siRNA for RhoA, Rac1, Cdc42 and compared to Scramble (SiScr). Being dependent on p21, cell migration, stress fiber number (SF), vinculin positive contacts, fibronectin matrix deposition and VEGF synthesis and immobilization into matrix are evaluated by way of RT-PCR and image analysis. We find that migration of SiScr cells increases in µg (+20%, $p<0.05$) as consequence of reduced adhesion (-35%, $p<0.01$) and increased polarity. SiRhoA in 1g group recapitulates most of SiScr cells µg-induced effects on focal adhesion area (-30%, $p<0.01$) and migration (+25%, $p<0.05$). SiRhoA does not induce major alteration in µg compared to µg SiScr and 1g SiRhoA. Interestingly, SiRac1 and SiCdc42 in 1g are not able to significantly increase SF and focal adhesion size while they increase both parameters in µg. SiRac cells in µg present an increased SF (+20%, $p<0.05$), matrix deposition (+25%, $p<0.01$) and a reduced migration (-40%, $p<0.01$). We previously showed (ASBMR 2006, #1274) that matrix-bound VEGF (VEGFm) expression is conditioned by a mechanically-increased SF. Whatever the silencing no change in basal levels of soluble (VEGFs) or VEGFm was seen in 1g. In µg, we observe that VEGFs/ VEGFm ratio is greatly in favor of VEGFs in SiSrc. SiRac1 and SiCdc42 in µg reduce significantly VEGFs, correcting VEGF ratio. All together SiRac1 and SiCdc42 may have activation effects on RhoA not seen in 1g leading to increased adhesion, matrix deposition and correction of VEGF alternative splicing thus counteracting µg-induced effects.

Disclosures: ***A. Guignandon****, None.*
This study received funding from: European Space Agency (ESA) and Centre National d'Etudes Spatiales (CNES).

SA012

See Friday Plenary number F012.

SA013

Deciphering the Cellular Defects of Osteoblasts in Microgravity. N. Nabavi*. University of Toronto, Toronto, ON, Canada.

Gravity has been shown to be essential for biological events occurring in organisms on earth. It is also known that major physiological changes occur in bone during space flights, however, the intracellular changes have not been analyzed in details due to the difficult nature of these studies. The goal of this project was to understand the molecular mechanisms by which bone loss occurs during space flights using an elaborate closed culture system enabling cell proliferation. In this study, we investigated the cellular mechanism of bone loss in microgravity conditions by first determining whether osteoblasts exhibit impaired differentiation and/or function in microgravity environments compared to ground controls. Mature osteoblasts grown in microgravity environments were fixed and immunostained with antibodies. Samples were analyzed post-flight using epi- and confocal microscopy in order to determine whether the actin and microtubule (MT) cytoskeletons as well as the associating regulating proteins show differential organization and stability in osteoblast cells during spaceflight compared to ground controls. Focal adhesion proteins as well as apoptosis were examined in osteoblasts grown in microgravity versus ground control. We observe a marked impairment of cytoskeletal organization and cell adhesion which correlates with increased cell death in osteoblasts grown in space. These findings may have important relevance for astronauts participating in extended flights, as well as relevance for bone-wasting disorders, including disuse osteoporosis.

Disclosures: ***N. Nabavi****, None.*
This study received funding from: The Canadian Space Agency.

SA014

See Friday Plenary number F014.

SA015

Microporosity In Beta-TCP Ceramics May Be Detrimental to Mesenchymal Stem Cell Survival and Osteoblastic Differentiation. J. Isaac*[1], J. Hornez*[2], D. Jian*[3], M. Descamps*[2], C. Chauveau*[1], P. Hardouin[1], D. Magne*[1]. [1]Cellular and Molecular Biology EA2603, ULCO/Lille University, Boulogne/Mer, France, [2]LMP, Université de Valenciennes et du Hainaut Cambrésis, Maubeuge, France, [3]Orthopaedic Surgery, Shanghai Jiaotong University Sixth People Hospital, Shanghai, China.

Aims of the present study were to determine whether microporosity in beta-tricalcium phosphate (b-TCP) ceramics is beneficial or detrimental to human mesenchymal stem cells (MSCs), for the development of hybrid constructs for bone repair. MSCs, either purified or contained in bone marrow stromal cells, were seeded on ceramics with 0%, 25%, or 45% microporosity and cultured from 18 hours, 7 days, 14 days or 21 days, in an osteogenic medium consisting of DMEM with 10% FCS supplemented with 10^{-8} M vitamin D3, 50 µM ascorbic acid and 10 mM beta-glycerophosphate. Cell adhesion and viability were measured by cell counting, and with MTS and LDH assays. Osteoblastic differentiation was evaluated by measuring the activity of alkaline phosphatase (ALP) by the method of Lowry and by quantifying osteocalcin secretion by ELISA. Results indicated that whereas microporosity had no effect on cell adhesion, it increasingly inhibited cell viability in a rate and time-dependent manner. In addition, early and late osteoblastic differentiation appeared stimulated on non microporous b-TCP ceramics. Indeed, ALP activity and osteocalcin secretion were always decreased by the more microporous ceramics. Results of this in vitro study therefore reveal an unexpected negative role for microporosity in the osteoblastic differentiation of human mesenchymal stem cells.

Disclosures: ***D. Magne****, None.*

SA016

JNK Activation Is Involved in Tumor Necrosis Factor-α-stimulated Smurf1 Expression. H. Lee*[1], T. Yi*[2], H. Ryoo[1], K. Woo[1], G. Kim*[1], J. H. Baek[1]. [1]Department of Cell and Developmental Biology, Seoul National University School of Dentistry, Seoul, Republic of Korea, [2]Clinical Research Center, INHA University Hospital, Incheon, Republic of Korea.

Tumor necrosis factor-α (TNF-α) is a pro-inflammatory cytokine that induces local and systemic bone loss through the stimulation of bone resorption as well as through the inhibition of bone formation. Although previous report has shown that TNF-α stimulated Smurf1 expression, the signal pathways involved in TNF-α-induced Smurf1 transcription have not been clarified yet. Therefore, we were to examine the intracellular signaling pathways involved in TNF-α-mediated Smurf1 expression in C2C12 cells. As reported previously, TNF-α stimulated Smurf1 mRNA and protein expression in C2C12 cells. Cycloheximide treatment did not exert any effects on TNF-α-induced Smurf1 mRNA expression, indicating that Smurf1 induction is a direct effect. Although TNF-α-inhibited ALP activity was partially rescued by blocking of NF-κB activation as well as by c-Jun N-terminal kinase (JNK) inhibition, Smurf1 induction was suppressed only by JNK inhibition but not by NF-κB inhibition. This result suggests that JNK activation is involved in TNF-α-mediated Smurf1 induction.

Disclosures: ***J.H. Baek****, None.*

SA017

See Friday Plenary number F017.

SA018

TGIF Regulation of Bone Mass: Impaired Osteoblast Differentiation. M. K. Crook[1], K. Mohammad[1], L. Bartholin*[2], A. Carver*[3], L. Suva[3], J. Chirgwin[1], D. Wotton*[2], T. Guise[1]. [1]Internal Medicine, University of Virginia, Charlottesville, VA, USA, [2]Biochemistry and Molecular Genetics, University of Virginia, Charlottesville, VA, USA, [3]Orthopedics, University of Arkansas, Little Rock, AR, USA.

TG Interacting factor (TGIF) is a transcriptional corepressor of TGF-beta and retinoic acid (RA) actions. TGIF mutations occur in holoprosencephaly (HPE), a defect in craniofacial and forebrain development, also caused by mutations at three steps in the Sonic Hedgehog (SHH) pathway: SHH, Ptc-1 and Gli-2. Zebrafish lacking TGIF have low RA-activating enzyme, aldehyde dehydrogenase 1a2 (aldh1a2) and degrading enzyme cyp26a1, suggesting that TGIF may regulate RA activity gradients. Aldh1a2-/- mice have diminished SHH signaling and severe skeletal abnormalities. The SHH promoter is RA-responsive, and TGIF may act on the skeleton via altered RA activity regulating SHH expression. *Tgif*-/- mice show growth retardation, domed skulls, twisted noses, and defects in vertebrae, ribs and sternum. These mice had significantly less trabecular bone by DEXA, microCT and histomorphometry. Low bone mass was accompanied by increased osteoclast activity and reduced osteoblast number and bone formation rates. Osteoblast progenitors, CFU-OBs, were decreased in bone marrow from *Tgif*-/- compared to wild-type (WT) mice. Primary osteoblasts from *Tgif*-/- vs WT mice had increased expression of the early marker Col1A ($p<.01$), and reduced expression of two late markers, osteocalcin ($p<.05$) and bone sialoprotein ($p<.01$), suggesting multiple effects of TGIF on the osteoblast lineage.

We previously showed that TGF-beta inhibition did not correct the low bone mass in *Tgif*-/- mice-beta. Here we focus on RA signaling. Aldh1a2 mRNA was 30% lower in *Tgif* -/- osteoblasts ($p<0.05$) compared to WT in an initial experiment. Cyp26a1 was increased by 30% ($P<.01$), suggesting that TGIF may determine RA gradients. SHH induces osteoblastogenesis and mineralization by increasing Gli-2 expression. In one of two experiments, Gli-2 mRNA concentration was decreased in untreated *Tgif*-/- osteoblast ($p=.0033$) compared to WT. Diminished SHH signaling may contribute to impaired differentiation in stimulated *Tgif* -/- osteoblasts.

The actions of RA and SHH signaling are best understood during embryogenesis. The epistatic relationship of these two pathways post-developmentally and whether they act primarily on adult mesenchymal stem cell differentiation are presently unclear. TGIF may play a role in adult bone remodeling by modulating these pathways.

Disclosures: ***M.K. Crook****, None.*

SA019

Conditional Disruption of *Pkd1* in Osteoblasts Results in Osteopenia Due to Direct Impairment of Osteoblast-Mediated Bone Formation. Z. Xiao, S. Zhang*, L. Cao*, R. Wu*, L. Quarles. The Kidney Institute, Kansas University Medical Center, Kansas City, KS, USA.

Polycystin-1 (PKD1) is a highly conserved, multi-domain membrane protein widely expressed in various cell types. Mutations of human *PKD1* cause Autosomal Dominant Polycystic kidney disease, however, the biological functions of PKD1 in other tissues remain poorly defined. We recently reported that *Pkd1* is highly expressed in bone and that the *Pkd1*^{m1Bei} mouse, which has an inactivating mutation of *Pkd1*, develops osteopenia and impaired osteoblastic differentiation. To determine whether Pkd1 directly functions in osteoblasts to regulate bone mass, we generated mice with osteocalcin (*Oc*)-promoted selective inactivation of *Pkd1* in osteoblasts. Transgenic *Oc*-Cre mice were crossed with *Pkd1*$^{m1Bei/+}$ mutant mice to create double heterozygous Cre/+; *Pkd1*$^{m1Bei/+}$ mice, then *Oc*-Cre/+; *Pkd1*$^{m1Bei/+}$ mice were crossed with homozygous floxed *Pkd1* (*Pkd1*$^{flox/flox}$) mice to generate control mice (*Pkd1*$^{flox/+}$), conditional *Pkd1* heterozygous mice (*Oc*-Cre-*Pkd1*$^{\Delta flox/+}$), and conditional *Pkd1* null mice (*Oc*-Cre-*Pkd1*$^{\Delta flox/m1Bei}$). Diagnostic PCR demonstrated that Cre-mediated recombination (*Pkd1*$^{\Delta flox}$) occurred exclusively in bone, whereas non-skeletal tissues retained the floxed *Pkd1* allele (*Pkd1*^{flox}). Compared to control mice, the conditional deletion of *Pkd1* from osteoblasts resulted in a gene-dose dependent reduction in bone mineral density (BMD; 10 verse 18%) compared to controls at 3 months-of-age. In addition, μCT analyses found that bone volume (BV/TV, %) and cortical thickness (Ct.Th., mm) were reduced in *Oc*-Cre-*Pkd1*$^{\Delta flox/+}$ (25.5±2.5 % and 0.241±0.009 mm) and *Oc*-Cre-*Pkd1*$^{\Delta flox/m1Bei}$ (18.8±1.6 % and 0.211±0.005 mm) compared with the age-matched control littermates (33.7±2.1 % and 0.267±0.005 mm). Mineral apposition rates were also reduced proportionate to *Pkd1* gene dose (1.28±0.31, 0.83±0.13, and 0.45±0.07 μm/day in control, *Oc*-Cre-*Pkd1*$^{\Delta flox/+}$ and *Oc*-Cre-*Pkd1*$^{\Delta flox/m1Bei}$, respectively). Real-time RT-PCR analyses also revealed a conditional *Pkd1*-gene dosage effect on osteoblast-related genes expressions, including *Runx2*-II, *Osteocalcin*, *Osteopontin*, and *bone sialoprotein*. Thus, the selective deletion of *Pkd1* from osteoblasts postnatally results in defective osteoblast-function and osteopenia. These findings indicate that Pkd1 functions in osteoblasts to regulate bone formation.

Disclosures: ***Z. Xiao****, None.*
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SA020

See Friday Plenary number F020.

SA021

Noncanonical Wnt Signaling Is Involved mir-206 Expression in Osteoblastic Differentiation. M. Sato*[1], M. Nashimoto*[2], D. Kanayama*[1], Y. Yawaka*[3], M. Tamura[1]. [1]Biochemisrty and Molecular Biology, Grad. Sch. Dent. Med., Hokkaido University, Sapporo, Japan, [2]Department of Applied Life Sciences, NUPALS, Niigata, Japan, [3]Dentistry for Children and Disabled Person, Grad. Sch. Dent. Med., Hokkaido University, Sapporo, Japan.

Wnt signaling plays a role in the developing skeletal system. However, the exact mechanisms by which Wnt signaling regulates bone remodeling remain to be elucidated. Our previous studies demonstrated that an interaction between β-catenin and Smad was crucial for Wnt-mediated regulation of BMP-2 responsive gene expression, and that BMP-2 up-regulated Wnt-induced osteoprotegerin (OPG) expression. MicroRNAs (miRNAs) are small non-coding RNAs that are emerging as important post-transcriptional gene regulators. Many miRNA are expressed in a tissue-specific manner, suggesting a role of the miRNA in the specification of the tissue during differentiation. In this study, we investigated the regulation of miRNA expression by Wnt signaling in osteoblastic differentiation. We cultured Wnt3a or Wnt5a stably over-expressing mesenchymal pluripotent C2C12 cell lines and examined levels of miR206 expression using TaqMan MicroRNA Assays. In C2C12 cells, miR-206 was expressed, while its expression was dramatically reduced in Wnt5a over-expressing C2C12 (Wnt5a-C2C12) cells. In contrast, miR-206 expression was similar in Wnt3a-C2C12 cells. These results indicated that non-canonical Wnt mediated signaling regulates miR-206 gene expression, and this is the first study linking miRNA expression to Wnt signaling. The miR-206 expression levels were also down-regulated by treatment with BMP2 in C2C12 cells. To further investigate the function of miR-206, we have examined over-expression of miR-206 or silencing of miR-206 by sgRNA (tRNaseZL-utilizing gene silencing method). Transfection of miR-206 reduced cell number in Wnt5a-C2C12 cells, indicating that miR-206 might regulate cell proliferation in these cells. RT-PCR analysis indicated that Id-1 expression was reduced by transfection of miR-206. These results show that miR-206 is a target gene for non-canonical Wnt signaling, and that its link is mediated by a novel mechanism by which miRNAs regulate cell proliferation and osteoblastic differentiation.

Disclosures: ***M. Tamura****, None.*

SA022

Accelerated Fracture Callus Remodeling and Membranous Bone Healing in STAT1 Deficient Mice. K. Tajima*[1], H. Takaishi[1], N. Ota[1], N. Kosaki[1], T. Tomonda*[1], M. Yoda*[2], J. Takito*[1], M. Matsumoto*[2], Y. Toyama*[1]. [1]Department of Orthopaedic Surgery, Keio University School of Medicine, Tokyo, Japan, [2]Spine and Spinal Cord Disease, Keio University School of Medicine, Tokyo, Japan.

Signal transducer and activator of transcription 1 (STAT1), originally identified as a signal transducing molecule in the interferon (IFN) pathway, participates not only in immune responses but also acts directly on ostoclast precursor cells and mesenchymal cells. Bone fracture triggers a steady inflammatory cascade of bone regeneration and this reparative process consists of a variety of molecular and cellular events. Although various growth factors and cytokines that participate in fracture healing have been identified, the precise mechanism behind these processes has not been fully understood. Here, we generated a fracture model using STAT1 knock out (KO) mice to elucidate the role of STAT1 in fracture healing.

A transverse osteotomy was performed at the middle of the tibia of 8-week-old male STAT1 KO and wild type (WT) mice. The fracture site was stabilized by inserting a inner pin of a 23-gauge spinal needle intramedullary. Soft X-ray and micro CT scanning were

performed at 1, 2, 3, 4, 6, and 10 weeks after surgery. There was no difference in the rate of callus formation and bone union between STAT1 KO and WT until post-fracture-week (PFW) 4. But the cross sectional area of the fracture site after bone union significantly decreased in KO mice (1.48±0.39 mm^2 at PFD6 and 1.35±0.32 mm^2 at PFW10) compared to WT (2.41±0.27 mm^2 at PFW6 and 2.32±0.18 mm^2 at PFW10), suggesting the accelerated callus resorption and bone remodeling in STAT1 KO. To examine the membranous ossification in STAT1 KO mice, we next generated a cortical defect model in tibiae. Soft X-ray and histological analysis were performed at PFW1, 2, 3, and 4. STAT1 KO mice showed the rapid recovery at PFW2 and 3. The number of stromal cells existed in the defect area was the same between WT and STAT1 KO.
It is known that the skeletal phenotype in STAT1 KO mice exhibits a high bone turnover typed osteosclerosis due to accelerated RANKL-dependent osteoclastogenesis and the enhanced calcification by osteoblasts. The enhanced calcification of STAT1 KO osteoblasts is explained by the release of inhibition of Runx2 function by STAT1. Since there was no significant difference in callus formation in two groups, we assumed that the STAT1 attenuated the callus remodeling and the membranous ossification in fracture healing. Further investigation of the STAT1 signaling *in vitro* will be presented.

***Disclosures:* K. Tajima**, *None.*

SA023

Characterization of Jun Activation-Domain Binding Protein (Jab1)-Interacting Motif in LIM Mineralization Protein-1. S. Sangadala, Y. Liu, M. Viggeswarapu, L. Titus, S. Boden. Orthopaedics, Atlanta VA Medical Center, Atlanta, GA, USA.

Members of the transforming growth factor β (TGFβ) superfamily have important effects on osteoblast differentiation and bone formation. We have shown previously that LIM Mineralization Protein-1 (LMP-1) enhances the efficacy of BMP-2, a member of the TGFβ superfamily, in cultured mesenchymal stem cells by the association of LMP-1 with Smurf1. Additionally, we report here that LMP-1 associates with Jab1, a protein also involved in protein degradation pathways like Smurf1. We screened a bone marrow library using the yeast-2-hybrid system. Sequencing and database matching of positive clones identified Jun activation domain binding protein 1 (Jab1) as a candidate binding partner for LMP-1. Immunoprecipitations demonstrated that Jab1 was found in complexes with LMP-1.
Jab1 is also known as the 5th subunit of the COP signalosome exhibiting homology to the 26S proteasomal lid complex. Jab1 binds to Smad4, Smad5 and Smad7, key intracellular signaling molecules of the TGFβ superfamily, and results in ubiquitination and/or degradation of these Smads. We confirmed a direct interaction of Jab1 with LMP-1 using recombinantly expressed proteins in slot-blot binding assays. In these studies, we also identified interaction of Smad1 with Jab1, in addition to Smads4, 5, and 7 as reported in literature. We hypothesized that LMP-1 binding to Jab1 prevents the binding and subsequent degradation of these Smads causing increased accumulation of osteogenic Smads in cells. To test this hypothesis we overexpressed LMP-1 in cells, separated the nuclear proteins by SDS-PAGE, and probed blots with Smad4 specific antibody. We demonstrated a 2-fold increase in the amount of Smad4 detected in LMP-1 treated cells compared with untreated cells. The phosphorylated receptor Smads1, 5 or 8 oligomerize with Smad4, enter the nucleus and induce osteogenic genes in the BMP pathway. An increase in nuclear Smad4 is an indicator of enhancement of this pathway.
We identified a motif in LMP-1 that is predicted to interact with Jab1 based on the MAME/MAST sequence analysis of several cellular signaling molecules that are known to interact with Jab-1 such as p27 (a cyclin-dependent-kinase inhibitor), Leukocyte functional antigen-1, lutropin/choriogonadotropin receptor, c-jun, Smad4, p53 and psoriasins. We further mutated the potential key interacting residues in LMP-1 and showed loss of binding to Jab1 in binding assays *in vitro*. Further studies are underway to establish biological relevance of LMP-1 and Jab1 interaction in enhancing the levels of osteogenic Smads resulting in potentiation of signaling by members of the TGFβ superfamily.

***Disclosures:* S. Sangadala**, *None.*

SA024

See Friday Plenary number F024.

SA025

PTH/PTHrP Mediated Stimulation of Osteoblast Differentiation Involves Epac-Rap1 Dependent Processes. N. S. Datta[1], N. J. D'Silva*[2], L. K. McCauley[2], A. B. Abou-Samra[1]. [1]Internal Medicine, Wayne State University, Detroit, MI, USA, [2]Periodontics and Oral Medicine, University of Michigan, Ann Arbor, MI, USA.

The mechanisms underlying the crosstalk between the cAMP and mitogen- activated protein kinases (MAPKs or ERKs) and the involvement of the MAPK pathway in the actions of PTH and PTHrP on osteoblast differentiation and mineralization are not completely understood. Our previous studies suggested involvement of ERK and cyclin D1 in the proliferative and differentiative actions of PTH and PTHrP; expression of cyclin D1 was dependent on ERK activity in MC3T3 osteoblastic cells. The mechanisms involved in the activation or inhibition of ERK signaling by cAMP include activation of the small G protein Ras and the protein kinase Raf-1 as well as the monomeric G protein Rap1 (GTPase Rap1) which preferentially activates B-Raf. cAMP can modulate ERKs via the Epac/Rap1/B-Raf pathway in a PKA- and Ras- independent manner. Although studies *in vivo* suggest that the anabolic actions of PTH and PTHrP are mediated by cAMP it is not known if Epac, Rap1 and B-Raf signaling pathway is involved. To examine the involvement of Rap1 signaling pathway on the growth of MC3T3-E1 osteoblastic cells, the cells were synchronized by switching them to media without serum for 36-48h to render them quiescent and then treated with or without PTHrP (100 nM) in low serum (2%) for 30 min-5h and harvested. Differentiation was induced with ascorbic acid for 7 days; and the cells were then treated with vehicle or PTHrP for 30 min- 5h before harvesting. Total cellular proteins and RNA were isolated. Using a pull-down assay, active GTP bound-Rap1 was up-regulated following SDS PAGE and Western blot analyses in differentiated MC3T3-E1 osteoblastic cells. Real-time PCR analysis demonstrated induction of Rap1B transcription ($p<0.01$) in these cells following PTHrP treatment. Ectopic ossicles, an engineered bone growth model generated with bone marrow stromal cells from C57BL6 mice implanted in nude mice, showed an increase in bone formation and increased Rap1B transcription ($p<0.01$) following 2 weeks of daily PTH treatment (40 μg/kg; sc) *in vivo*. These studies show that activation of the PTH/PTHrP receptor in the osteoblastic cells increase the expression of Rap1B. The increased Rap1B provides an alternate signaling mechanism for the expression of the anabolic action of PTH and PTHrP in the differentiated osteoblasts.
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This study received funding from: NIH.

SA026

See Friday Plenary number F026.

SA027

Ahnak Regulates Calcium Signaling and ATP Release in Osteoblasts in Response to Mechanical Stimulation. Y. Shao, V. P. Fomin*, M. C. Farach-Carson, R. L. Duncan. Biological Sciences, University of Delaware, Newark, DE, USA.

We have found that ahnak, a 700kDa scaffold protein, interacts with the auxiliary β_2 subunit of the $Ca_v1.2$ cardiac L-type voltage sensitive calcium channel (LVSCC) and appears to anchor these channels to the actin cytoskeleton. Western blot analysis and immunohistochemistry demonstrated that ahnak was primarily expressed at the plasma membrane of mouse osteoblasts, with relatively lower intracellular distribution. In addition to the structural function of ahnak, we postulate that ahnak can regulate LVSCC activity and, as a result, alter ATP release in osteoblasts in response to fluid shear. Using siRNA strategy directed at ahnak in MC3T3-E1 osteoblasts, we were able to suppress ahnak protein levels by approximately 80% of vector transfected controls. The intracellular Ca^{2+} increase in response to mechanical stimulation in MC3T3-E1 cells was significantly reduced by 70% in response to ahnak protein knockdown indicating a possible role of ahnak in the regulation of Ca^{2+} signaling. We have previously shown that this increase in Ca^{2+} through LVSCC activation was responsible for fluid shear-induced ATP release from MC3T3-E1 cells. Although ahnak protein knockdown had no effect on basal ATP release in static cells, fluid shear-induced ATP release was significantly attenuated in siRNA transfected cells ($p<0.01$). Interestingly, quinacrine staining indicated a marked accumulation of acidic vesicles (mostly ATP rich vesicles) in siRNA transfected cells in static conditions. While we have shown that shear induces polymerization of actin filaments, knockdown of ahnak protein with specific siRNA did not influence the structures of either actin filaments or microtubule cytoskeleton at static conditions. Microtubule filaments are disassembled in response to fluid shear untreated osteoblasts. However the microtubule structure was kept intact in ahnak siRNA transfected cells, suggesting that ahnak may participate in the microtubule-mediated vesicular ATP transportation and release. Collectively, our investigation revealed that ahnak functions in Ca^{2+} signal regulation in bone cells independently of the actin-based cytoskeleton. Ahnak protein was clearly involved in the ATP release induced by mechanical stimulation. Furthermore, Western analyses demonstrate that ahnak protein levels are rapidly reduced in MC3T3-E1 cells in response to fluid shear, suggesting that loss of this protein may be involved in the loss of mechanosensitivity in response to continued loading.

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SA028

Integrin-associated Protein Upregulates Osteogenesis-related Transcription Factors via TGF-β and BMP Pathways. K. Ikeda*[1], K. Shimada[1], K. Kawamoto*[1], M. Maeno[2], K. Ito*[1]. [1]Department of Periodontology, Nihon University, Tokyo, Japan, [2]Department of Oral Health Sciences, Nihon University, Tokyo, Japan.

To understand the mechanism of developing bone leads to open new frontiers in bone regeneration. To analyze the molecular events that occur in the developing mandible, we examined the expression of 8803 genes from samples taken at different time points during rat postnatal mandible development using cDNA microarray. We demonstrated that gene expression of Integrin-associated protein (IAP, also known as CD47) changed markedly.
Transforming growth factor (TGF)-β superfamily, including bone morphogenetic proteins (BMPs), play a specific role in developing bone. TGF-β and BMPs induce receptor-regulated Smads (R-Smads); Smad2, 3 or Smad1, 5, 8, respectively. The R-Smads form complexes with the common-partner Smad, which is Smad4 in mammals. These complexes translocate and accumulate in the nucleus and regulate the transcription of various target genes.
To analyze the function of IAP in the TGF-β and BMP signaling pathway, endogenous IAP was disrupted in rat osteoblastic cell line ROS 17/2.8 using a 19 nucleotide siRNA and examined its effectiveness in silencing IAP expression. The gene expression of osteogenesis-related transcription factors, including Runx2, Msx2, Dlx5 and Osterix was determined by real-time reverse-transcribed PCR. Moreover, the protein levels of Smad4, Smad1, phospho-Smad1, Smad2, phospho-Smad2 were evaluated using SDS-PAGE and Western blot analysis.
The efficiency of silencing IAP mRNA level was approximately 70%. The expression of Runx2, Msx2, Dlx5 and Osterix was markedly increased when IAP was silenced. The protein levels of Smad4, phospho-Smad1, phospho-Smad2 were increased dose dependently. Our results suggest that IAP regulates osteogenesis-related transcription factors via TGF-β and BMP pathways.

***Disclosures: K. Ikeda**, None.*
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SA029

See Friday Plenary number F029.

SA030

Lithocholic Acid Downregulates the Effects of Vitamin D_3 on Primary Human Osteoblasts. S. Ruiz-Gaspà*[1], A. Enjuanes*[1], P. Peris[1], A. Martinez-Ferrer*[1], M. J. Martinez de Osaba*[1], L. Alvarez*[1], A. Monegal*[1], A. Combalia*[2], B. Gonzalez*[1], N. Guanabens[1], A. Pares*[3]. [1]Metabolic Bone Diseases Unit, Hospital Clinic, IDIBAPS, CIBEREHD, Barcelona, Spain, [2]Department of Orthopaedics, Hospital Clinic, IDIBAPS, CIBEREHD, Barcelona, Spain, [3]Liver Unit, Hospital Clinic, IDIBAPS, CIBEREHD, Barcelona, Spain.

Osteoporosis is a complication of chronic cholestasis, related to its severity and duration. Cholestasis is characterized by deficient biliary secretion of total and conjugated bilirubin and bile acids, with high circulating levels. Among bile acids, lithocholic acid has particular significance because it has potential toxic effects and on the other hand has been described as an agonist of vitamin D receptor. Therefore, this study was addressed to analyse the effect of lithocholic acid on survival and on pathways of vitamin D metabolism and cell maturation on primary human osteoblasts.
Bone cell cultures were established from trabecular bone specimens of 10 patients undergoing hip replacement for osteoarthritis. Experiments with human osteoblastic cell cultures were performed using DMEM with or without fetal bovine serum (FBS) or human albumin, and at different lithocholic acid concentrations (10^{-6}M, 10^{-5}M, 10^{-4}M and 10^{-3}M) and times (6, 24, 48 and 72 hours) with or without vitamin D_3 (10^{-7}M). Cell survival was determined by cell proliferation reagent WST-1. Vitamin D 24-hydroxylase (*CYP24A*), bone gamma-carboxyglutamate protein (*BGLAP)* and osteoprotegerin (*TNFRSF11B)* gene expression were quantified by real time PCR and used as indicators of vitamin D metabolism, osteoblast maturation and osteoclast differentiation and activation, respectively.
Lithocholic acid with or without FBS or albumin at 10^{-3}M and 10^{-4}M, respectively had a toxic effect evidenced by a dramatic decrease in cell survival. All the following experiments were performed with lithocholic acid below these lethal concentrations. Lithocholic acid increased *CYP24A* (p=0.02) and *BGLAP* (p=0.007*)* mRNA expression in cell cultures with no changes on *TNFRSF11B* expression, effects which were significantly lower (*CYP24A:* - 96% and *BGLAP:* - 92%) than those observed with vitamin D_3. Moreover, lithocholic acid at all time points and conditions significantly downregulated the effect of vitamin D_3 on *CYP24A* and *BGLAP* gene expression by -72% and -74%, respectively.
In conclusion, lithocholic acid decreases the stimulatory effect of vitamin D_3 on *CYP24A* and *BGLAP* expression in primary human osteoblasts. These results may explain the potential deleterious effects of retained toxic bile acids on bone formation in patients with chronic cholestasis.

***Disclosures: S. Ruiz-Gaspà**, None.*

SA031

The Interplay Between BMP and TGF-β Signaling in Osteoblast Differentiation. D. J. J. de Gorter*[1], R. L. van Bezooijen*[2], C. W. G. M. Löwik*[2], P. ten Dijke*[1]. [1]Molecular Cell Biology, Leiden University Medical Center, Leiden, Netherlands, [2]Endocrinology, Leiden University Medical Center, Leiden, Netherlands.

Transforming Growth Factor-β (TGF-β) is a pleiotropic cytokine implicated in the control of proliferation, migration, differentiation, and survival of many different cell types. In the skeleton, TGF-β is one of the most abundant cytokines and plays a major role in its development and maintenance by affecting both cartilage and bone metabolism. Both positive and negative effects of TGF-β on bone formation have been reported, but the exact molecular mechanisms underlying these effects remain to be established. Among others, TGF-β modulates signaling induced by Bone Morphogenetic Proteins (BMPs), which are required for skeletal development and maintenance of adult bone homeostasis, and play an important role in fracture healing.
Although TGF-β is generally believed to inhibit BMP-induced osteoblast differentiation, we found that pharmacological inhibitors against the TGF-β type I receptor ALK5 impaired BMP-induced alkaline phosphatase (ALP) activity in the murine KS483 mesenchymal and C2C12 pre-myoblast cell lines. In addition, these ALK5 inhibitors reduced BMP6 stimulated mineralization of KS483 cells. Conversely, co-stimulation with BMP6 and TGF-β3 further increased ALP activity and mineralization of KS483 cells compared to BMP6 alone. This was not only observed in combination with BMP6, but also with BMP2 and BMP7. In C2C12 cells, TGF-β3 could inhibit but also under certain conditions stimulate BMP-induced ALP activity. BMP6-induced activation of a BMP-Smad-dependent luciferase reporter was, however, always inhibited by TGF-β3, suggesting that the stimulatory effect of TGF-β3 is not due to increased BMP-Smad activity.
In conclusion, besides acting as an inhibitor, TGF-β can also stimulate BMP-induced osteoblast differentiation. Characterization of the interplay between BMP and TGF-β signaling and the conditions in which TGF-β inhibits or stimulates BMP-induced osteoblastic differentiation may provide new insights into the molecular mechanisms that regulate bone formation and how this may be modulated.

***Disclosures: D.J.J. de Gorter**, None.*

SA032

See Friday Plenary number F032.

SA033

PTH Induces COX-2 in MC3T3-E1 Osteoblasts via cAMP-PKA and Calcium-Calcineurin Pathways. H. Huang*[1], D. Chikazu[2], O. Voznesensky*[1], K. Dodge-Kafka*[3], H. Drissi[4], C. Pilbeam[1]. [1]Department of Medicine, UCHC, Farmington, CT, USA, [2]Department of Oral and Maxillofacial Surgery, University of Tokyo, Tokyo, Japan, [3]Department of Cell Biology, UCHC, Farmington, CT, USA, [4]Department of Orthopedics, UCHC, Farmington, CT, USA.

Parathyroid hormone (PTH) is a major regulator of bone remodeling that is thought to have most of its effects via the cyclic adenosine monophosphate **(**cAMP)-protein kinase A (PKA) signaling pathway. PTH is also a potent inducer of cyclooxygenase-2 (COX-2) via the PKA pathway. The goal of this study was to identify cis-acting sites and trans-acting factors mediating the PTH induction of COX-2. We used osteoblastic MC3T3-E1 cells (MC-4 sub-clone) stably transfected with -371/+70 bp of the murine COX-2 promoter fused to a luciferase reporter. We made site-directed mutations in the promoter DNA in (1) the cAMP response element (CRE) at -57/-52 bp, (2) the activating protein-1 (AP-1) binding site at -68/-63 bp, (3) the nuclear factor of activated T-cells (NFAT) binding site at -77/-73 bp, and (4) both the AP-1 and NFAT sites (which comprise a consensus sequence for composite binding of NFAT and AP-1). Single mutation of the CRE, AP-1, or NFAT site decreased PTH-stimulated luciferase activity by about 60%, while mutation of the composite NFAT/AP-1 site reduced the PTH-stimulated activity by 90%. On electrophoretic mobility shift analysis (EMSA), PTH stimulated binding of phosphorylated CRE-binding protein (CREB) to an oligonucleotide spanning the CRE. PTH also increased binding of NFATc1, c-Fos and c-Jun to an oligonucleotide spanning the NFAT/AP-1 composite site. PTH did not increase binding of NFATc2, c3 or c4 to the composite site. EMSA competition experiments showed cooperative binding of NFATc1 and AP-1 proteins to the NFAT/AP-1 composite sequence, and the cooperativity depended more on NFAT than on AP-1 binding. Both PTH and forskolin, an activator of adenylyl cyclase, stimulated NFATc1 nuclear translocation. Phorbol myristate acetate, a protein kinase C (PKC) agonist, did not stimulate NFATc1 nuclear translocation. PKA inhibitors H-89 and KT-5720 inhibited PTH- or forskolin-stimulated COX-2 promoter activity 98 and 60%, respectively, while GF109203X, a specific PKC inhibitor, had no effect on either PTH- or forskolin-stimulated COX-2 promoter activity. Inhibition of the calcium-calcineurin-NFAT pathway by chelation of calcium or by calcineurin inhibitors reduced PTH- and forskolin-stimulated COX-2 promoter activity 60-80%. These results demonstrate a critical role for both the cAMP/PKA pathway and the calcium-calcineurin pathway in NFAT activation and PTH signaling in osteoblasts and suggest that cross-talk between these two pathways may play a role in other important effects of PTH in osteoblasts.

***Disclosures: H. Huang**, None.*
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SA034

See Friday Plenary number F034.

SA035

$P2Y_2$ Receptor Activation Regulates RhoA-Mediated Stress Fiber Formation in Osteoblasts. W. D. Yang[1], J. Gardinier*[2], S. Majumdar*[1], D. L. Randall[1]. [1]Biological Sciences, University of Delaware, Newark, DE, USA, [2]Biological Sciences, and Mechanical Engineering, University of Delaware, Newark, DE, USA.

Bone and osteoblasts rapidly lose their mechanosensitivity in response to continued loading, likely through adaption of cellular mechanism involved in the transduction of this signal. We postulate that cytoskeleton reorganization in response to load is one mechanism through which the desensitization of osteoblasts to load is incurred. Because we have shown that ATP is rapidly released from osteoblasts in response to mechanical load, we hypothesize that activation of purinergic signaling mediates the increase in actin organization. $P2Y_2$ receptor activation increases stress fibers formation in other cell types, therefore we examined the role of $P2Y_2$ in the regulation Rho-mediated stress fibers formation induced by mechanical stimulation in osteoblasts. In this study, we examined the effects of ATP and $P2Y_2$ on cytoskeleton formation, Rho activation and cell mechanical properties alterations in MC3T3-E1 cells subjected to 12 dynes/cm^2 fluid shear. When 100 μM ATP, ADP, BzATP or UDP were added to static cultures, ATP produced a significant change in actin stress fiber formation, mimicking the effects of shear. However, aside from a reduced effect of UDP on actin, the rest of the nucleotides tested had little effect on actin organization. Analyses of the effects of ATP and fluid shear on RhoA activation using a G-LISA kit indicated that both ATP and fluid shear increased Rho activation within minutes of stimulation, peaking at 15 min. To evaluate $P2Y_2$ function in Rho activation and stress fiber formation, MC3T3-E1 osteoblasts were transfected with $P2Y_2$ siRNA, which produced a 75% suppression of $P2Y_2$ protein 48 hours after transfection compared to vector controls. The MC3T3-E1 osteoblasts transfected with $P2Y_2$ siRNA failed to respond to ATP of fluid shear with an increase in either Rho activation or stress fiber formation. This was similar to the responses found with pretreatment of the cells with apyrase or RB-2 (P2Y2 specific inhibitor). Atomic force microscopy (AFM) found that cell stiffness was significantly increased after fluid shear or ATP treatment. In contrast, P2Y2 siRNA transfected cells did not increase cellular stiffness in response to these stimuli. This study suggests that $P2Y_2$ receptor activation by ATP is essential in the shear-induced, RhoA-mediated stress fiber formation, that this pathway regulates the mechanical properties of the osteoblast and that inhibitior of this pathway may reduce the loss of mechanosensitivity associated with extended loads.

***Disclosures:* *W.D. Yang**, None.*
This study received funding from: NIH.

SA036

Stable Isotopic Labeling of Amino Acids in Cultured Human Bone Marrow Stem Cells: Application to BMP2 Induced Wnt/β-catenin Signaling. J. Lee*[1], B. Kim*[1], J. Ahn*[1], H. Park*[1], E. Kim*[2], S. Park*[3], J. Kim*[1], E. Park*[4], J. Yoo*[2], J. Yates Ⅲ *[3], J. Cho*[1]. [1]Biochemistry, School of Dentistry, Kyungpook National University, Daegu, Republic of Korea, [2]Mass Spectrometer Development Team, Korea Basic Science Institute, Daejeon, Republic of Korea, [3]Cell Biology, The Scripps Research Institute, La Jolla, CA, USA, [4]Pathology and Regenerative Medicine, Kyungpook National University, Daegu, Republic of Korea.

Bone morphogenetic protein-2 (BMP2) is one of the most potent bone-inducing agents and promotes differentiation of osteoblasts from bone marrow stem cells (BMSC). However the potency of BMP2 varies in a species-specific manner. Especially, BMP2 induces alkaline phosphatase activity in human BMSCs is much less than rodents, although several other genes known to be steadily induced. Thus, there has been an increasing importance of discovering the unknown mechanism of BMP2 role in human BMSC. Here, we used SILAC(Stable isotope labeling with amino acids in cell culture) in combination with LTQ-FT-mass spectrometry not only to characterize the unknown mechanisms of human BMSC differentiation by BMP2 but also to find early differentiation markers. After tandem mass spectra were interpreted by SEQUEST using DTAselect2, ion chromatograms were quantified by Census program. In this analysis, 414 proteins of total 449 uniquely identified were successfully quantified with 79.21% quantification efficiency of peptides (2762 of 3487 total peptides). Of these, 12 were found to be increased more than 1.4-fold in BMP-2 stimulated cells. In addition, 18 proteins were detected only in heavy-labelled cells. Nine proteins were down-regulated less than 1.4-fold by BMP2 stimulation. Interestingly, β-catenin was only detected in heavy-labelled, BMP-2 stimulated cells. BMP2-increased β-catenin level in time-dependent and dose-dependent manner. BMP-2 stimulation also increased phospho-Gsk3β levels which are known to result in the increment of β-catenin. These results suggest that up-regulation of Wnt signaling in early time could potentiate or accelerate the capability of BMP2 induced osteoblast differentiation. In conclusion, our investigation that the SILAC combined MS-based analysis could provide the basis for the understanding of human BMSC differentiation to osteoblast mechanisms upon BMP2 stimulation.

***Disclosures:* *J. Lee**, None.*

SA037

High Concentrations of Hydroxytyrosol and Quercetin Antioxidants Enhance Adipogenesis and Inhibit Osteoblastogenesis in Mesenchymal Stem Cells. A. Casado-Díaz*[1], J. Anter*[2], R. Santiago-Mora*[3], L. Luque*[4], G. Dorado*[5], J. M. Quesada[3]. [1]Dpto. I+D, SANYRES XXI (Grupo PRASA), Córdoba, Spain, [2]Dep. Genética, Universidad de Córdoba, Córdoba, Spain, [3]Unidad de Metabolismo Mineral, Hospital Universitario Reina Sofía, Córdoba, Spain, [4]Dep. Química Analítica, Universidad de Córdoba, Córdoba, Spain, [5]Dep. Bioquímica y Biología Molecular, Universidad de Córdoba, Córdoba, Spain.

The increase of age-related oxidative stress is associated with aging and major chronic aging-related diseases, such as loss of bone mineral mass and osteoporosis. Therefore, natural antioxidants may be potentially useful to reduce the impact of the oxidative stress on the organism in general and the bone in particular. We have analyzed the effect of different concentrations of plant antioxidants (hydroxytyrosol and quercetin) on the differentiation of mesenchymal stem cells (MSC). Thus, MSC were induced to differentiate to osteoblasts, in the presence of 10-8 M dexamethasone, 0.2 mM ascorbic acid and 10 mM β-glycerolphosphate, or to differentiate to adipocytes, using 5 x 10-7 M dexamethasone, 0.5 mM isobutylmethylxanthine and 50 μM indometacine. The cultures were supplemented with hydroxytyrosol (10-4 and 10-6 M) or with quercetin (10-5 and 10-7 M). The expression of osteoblastogenesis and adipogenesis marker genes was analyzed by quantitative real-time PCR (QRT-PCR) after seven and 14 days of treatment. The alkaline phosphatase activity was measured on the cells induced to osteoblasts, and the formation of fat vesicles was monitored after 14 days of induction to adipocytes. We found that 10-4 M hydroxytyrosol and 10-5 M quercetin significantly enhanced adipogenesis, increasing the number adipocytes and the expression of adipogenic marker genes (ppar-γ2 and lpl). The other tested concentrations did not affect, or increased the adipogenesis to a lesser extent. On the other hand, the higher hydroxytyrosol and quercetin concentrations tested inhibited the studied osteoblastogenesis markers, mainly after seven days of induction. These data suggest that although low concentrations of hydroxytyrosol and quercetin may enhance the bone formation, they have the opposite effect at concentrations higher than 10-5 M. Such high concentrations promote the adipogenic instead of the osteoblastogenic differentiation of MSC, which may affect negatively the bone formation.

***Disclosures:* *A. Casado-Díaz**, None.*
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SA038

See Friday Plenary number F038.

SA039

Concentration of Connective Tissue Progenitors in Autologous Cancellous Bone Graft Enhances New Bone Formation in a Canine Femoral Defect Model. K. Kumagai*[1], J. Otonichar*[1], R. Rozic*[1], C. Boehm*[1], K. Powell*[1], A. Vasanji*[1], T. Saito*[2], G. F. Muschler[1]. [1]Orthopaedic Research Center, The Cleveland Clinic Foundation, Cleveland, OH, USA, [2]Department of Orthopaedic Surgery, Yokohama City University, Yokohama, Japan.

Objective: Osteogenic connective tissue progenitors (CTPs) in bone marrow and cancellous bone generate progeny expressing osteoblastic markers *in vitro* and contribute to new bone tissue *in vivo*. Autologous cancellous bone (ACB) is known as one of the best bone graft materials. ACB is biocompatible and provides on osteoconductive bone matrix. ACB also contains osteogenic CTPs. The purpose of this study was to define the distribution of osteogenic CTPs in ACB and the effect of impaction grafting on their delivery and efficacy in a canine femoral defect model.
Methods: Four identical 1.0 cm diameter and 1.0cm deep unicortical cylindrical defects for grafting were created in unilateral femur of eight mongrel dogs. After harvest of bone marrow by aspiration and cancellous bone by curetting from proximal humerus, four defects in each femur were treated with implantation of: 1) no graft, 2) bone marrow clot (BM), 3) non-compacted autologous cancellous bone (ACB1x) and 4) compacted autologous cancellous bone (3x starting volume, ACB3x) using a Latin square design. Animals were sacrificed four weeks after implantation and graft site in femur were harvested for analysis. Bone formation within defects was assessed by quantitative micro CT analysis and histological findings.
Results: Seventy percent of osteogenic CTPs were located on the trabecular surface. Therefore, impaction grafting concentrated CTPs by squeezing out marrow cells having lower CTP prevalence. The mean percent of bone volume (%BV) in ACB3x (12.9) was significantly greater than that in no graft (2.9) (p = 0.021). Although there was no statistical difference, %BV in BM (9.5) and ACB1x (9.4) was higher than that in no graft. When each defect was radially divided into three regions composed of the central third (0-1.5mm from defect center), the middle third (1.5-3mm from defect center) and the outer third (3-4.5mm from defect center), there were significant differences of %BV between ACB3x and no graft in the middle third and the outer third (p = 0.022, 0.027, respectively). Better bone formation was evident in the upper half of each defect.
Conclusions: This study demonstrated that impaction graft of autologous cancellous bone contains a higher concentration of osteogenic CTPs and results in greater new bone formation.

***Disclosures:* *K. Kumagai**, None.*

SA040

Use of α-smooth Muscle actin-GFP Transgene to Identify Periodontium Derived Osteoprogenitors. S. M. San Miguel*[1], H. Li[1], J. C. Igwe*[1], V. Ferrer[1], H. Aguila*[2], I. Kalajzic[1]. [1]Dept. of Reconstructive Sciences, Uni. of Conn. Health Center, Farmington, CT, USA, [2]Dept. of Immunology, Uni. of Conn. Health Center, Farmington, CT, USA.

Cells with osteoprogenitor potential are present within the periodontal tissues during development and in postnatal life. In previous studies we identified an α-SMA-GFP expressed in mesenchymal progenitor cells within the bone marrow stromal population and in adipose tissue. The purpose of this study is to define whether the α-SMA promoter would direct GFP expression to osteoprogenitor cells in the dental follicle and periodontal ligament. We have utilized a transgenic mouse model approach in which GFP expression is driven by an α-SMA promoter. Epifluorescence was evaluated in histological sections of mandibles derived from neonatal and 4-6 week old mice. Alpha SMAGFP expression was detected in the dental follicle, but not in mature bone. GFP+ cells were observed in apical regions of the root, the areas rich with vascularization. In addition transgene expression was evaluated in primary cultures derived from dental follicle (mDF) and periodontal ligament (mPDL). Strong expression of α-SMAGFP was observed during early stages of the culture and diminished as mineralization progressed.
Observation of GFP expression was complemented with analysis of osteogenic differentiation by analysis of RNA expression of bone markers, histochemical staining for alkaline phosphatase and detection of mineralized nodules by xylenol orange. An intense ALP activity and the presence of mineralized nodules followed two weeks of osteogenic induction. Expression of BSP and DMP-1 increased during osteogenic differentiation. We utilized flow cytometry (FACS) to determine the proliferative potential and cellular profile of freshly isolated or cultured α-SMA positive cells. FACS analysis revealed that freshly digested mDF cells expressed the mesenchymal markers Thy1 (40%) and Sca1 (8.79%). In vitro expansion enriched for an α-SMAGFP+ population of which 60% of cells were Thy1+ and Sca1+. The α-SMAGFP+ population exhibited high proliferative potential, while proliferation of α-SMAGFP- population was very limited. Our data suggest that the α-SMA promoter has the potential to identify a population of osteoprogenitor cells residing within the dental follicle and periodontal ligament that can differentiate into mature osteoblasts.

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SA041

See Friday Plenary number F041.

SA042

Mature Human Adipocytes differentiated from Bone Marrow Derived Mesenchymal Stem Cells. A. C. Niemeier[1], J. Prawitt*[2], M. Kassem[3], U. Beisiegel*[2], J. Heeren*[2]. [1]Orthopaedics, University Hospital Hamburg-Eppendorf, Hamburg, Germany, [2]IMB II: Molecular Cell Biology, University Hospital Hamburg-Eppendorf, Hamburg, Germany, [3]Endocrinology, University Hospital Odense, Odense, Denmark.

Bone marrow adipocytes and osteoblasts share common mesenchymal precursor cells. It is a generally accepted concept that factors exist which stimulate the differentiation process in favour of either osteoblastogenesis or adipogenesis, and that these may in part be mutually exclusive. In this context, it is likely that adipocytes and osteoblasts influence each other in a paracrine fashion. In order to identify such factors, there is a demand for suitable human cell models. Most research with regard to these questions has focused on osteoblast differentiation.
Here we describe the adipogenic differentiation of a bone marrow derived telomerase-immortalized human mesenchymal stem cell line (hMSC-Tert) that maintains numerous features of terminally differentiated adipocytes even after prolonged withdrawal of the peroxisome proliferator activated receptor gamma (PPARgamma) agonist rosiglitazone. Differentiated hMSC-Tert developed the characteristic monolocular phenotype of mature adipocytes. The expression of adipocyte specific markers was highly increased during differentiation. Most importantly, the presence of the PPARgamma agonist rosiglitazone was not required for the stable expression the adipocyte markers lipoprotein lipase, adipocyte fatty acid binding protein and perilipin on mRNA and protein levels. Adiponectin expression was post-transcriptionally down-regulated in the absence of rosiglitazone. Insulin sensitivity as measured by insulin-induced phosphorylation of Akt and S6 ribosomal protein was also independent of rosiglitazone.
In summary, we describe a novel model for mature human adipocytes derived from bone marrow mesenchymal stem cells. This model with its unique characteristics will be a valuable tool for the in vitro identification of secreted adipocyte-specific factors which influence osteoblastogenesis.

***Disclosures: A.C. Niemeier**, None.*

SA043

Enhanced Mitochondrial Biogenesis Contributes to Wnt induced Osteogenic Differentiation of C3H10T1/2 Cells. J. H. An*, J. Y. Yang*, S. W. Cho, J. Y. Jung*, H. Y. Cho, Y. M. Cho*, S. W. Kim, C. S. Shin. Department of Internal Medicine, Seoul National University College of Medicine, Seoul, Republic of Korea.

Background: Mitochondria play a key role in cell physiology including cell differentiation. We investigated the changes of mitochondrial biogenesis during Wnt induced osteogenic differentiation of murine mesenchymal C3H10T1/2 cells.
Methods: C3H10T1/2 cells were cultured in DMEM and osteogenic differentiation was induced by Wnt-3A conditioned medium (CM). Mitochondrial mass, mitochondrial membrane potential and intracellular reactive oxygen species were measured by flow cytometry analysis. Oxygen consumption rate was analyzed by high-resolution respirometry and mitochondrial (mt) DNA copy-number by real-time PCR.
Results: gnaling by Wnt-3A CM resulted in significant increase alkaline phosphatase (ALP) activities in C3H10T1/2 cells as expected. The increase in ALP activities were associated with significant increase in mitochondrial mass, mitochondrial membrane potential, intracellular reactive oxygen species, oxygen consumption rate and mtDNA copy number compared to the control group. Co-treatment with DKK-1 and WIF-1, both of which are Wnt inhibitors, abrogated the Wnt-3A-induced ALP activities as well as mitochondrial biogenesis markers. Moreover, inhibition of mitochondrial biogenesis by Zidovudine treatment resulted in significant inhibition of osteogenic differentiation as measured by ALP activities. Finally, removal of mitochondria from human osteosarcoma cell line (ρ_0 cells) by ethidium bromide treatment resulted lower ALP activity both in basal and Wnt-3A stimulated state compared to that in mitochondria-intact cells (ρ_+ cells).
Conclusion: Mitochodrial biogenesis is upregulated by Wnt signaling and this upregulation seems to contribute to the osteogenic differentiation of mouse mesenchymal C3H10T1/2 cells.

***Disclosures: J.H. An**, None.*

SA044

See Friday Plenary number F044.

SA045

The Comparison of the Effects of Zoledronates on the Differentiation of Human Bone Marrow and Amniotic Fluid Derived Mesenchymal Stem Cells. J. An*[1], I. Kim*[2], Y. Kim*[2], B. Joo*[3]. [1]Internal Medicine, Good Moonhwa Hospital, Busan, Republic of Korea, [2]Internal Medicine, Busan National University Hospital, Busan, Republic of Korea, [3]Center for Reproductive Medicine, Good Moonhwa Hospital, Busan, Republic of Korea.

Background: Mesenchymal stem cells (MSCs) were identified in various tissues including human bone marow and adipose tissue. Recently it has been known that amniotic fluid is rich source of fetal MSCs. Zoledronate is the most potent amino-bisphosphonate and act primarily by inhibiting osteoclast. But many reports show that zoledronate has anabolic action by influencing osteoblast differentiation. This study was aimed to compare the effects of zoledronates on the differentiation of MSCs derived from human bone marrow and amniotic fluid into osteoblast and adipocyte.
Method: Amniotic fluids were collected from 5 pregnanted women who underwent amniocentesis at the second trimester due to chromosomal analysis and adult male-bone marrows were obtained from 3 patients who underwent total hip arthroplasty. Zoledronate was treated to MSC derived from amniotic fluid and bone marrow to induce differentiation into osteoblsast and adipocyte. Osetoblast was confirmed by alkaline phosphatase (ALP) activity assay and characteristics of adipocyte was determined by sterol regulatory element binding protein 1(SREBP-1), fatty acid synthase (FAS), acetyl CoA carboxylase 1 (ACC1) activities.
Result: ALP activity tended to more prominent in MSCs derived from amniotic fluid than that of bone marrow. In osteogenic differentiation from amniotic fluid, ALP activitiy was positively correlated with concentration of zoledronate and it was significantly increased at 10^{-6}M concentration of zoledronate. However, in bone marrow, ALP activity was are negatively correlated with concentration of zoledronate. SREBP1 and ACC1 activities tended to be decreased but FAS activities tend to be increased in MSCs derived from both amnitic fluid and bone marrow.
Conclusion: These results suggest that amniotic fluid seems to be more rich source of MSCs to be diffrentiated into osteoblast and adipocyte than bone marrow. Zoledronates may influence differently on the differentiation of MSCs according to their origins.

***Disclosures: J. An**, None.*

SA046

Overexpression of Alpha-catenin in C3H10T1/2 Cells Increases Osteoblast Differentiation. D. Kim*[1], J. Yang*[2], J. Chung*[2], S. Cho*[2], S. Kim*[2], S. Kim*[2], C. S. Shin[2]. [1]Internal Medicine, Dankook University College of Medicine, Cheon-An, Republic of Korea, [2]Internal Medicine, Seoul National University College of Medicine, Seoul, Republic of Korea.

Alpha- and beta-catenin link cadherins to the actin-based cytoskeleton at adherens junctions and regulate cell-cell adhesion. Beta-catenin is also an important component of Wnt signaling pathway. Although roles of cadherins and canonical Wnt signaling in osteoblastic differentiation have been extensively studied, the role of alpha-catenin is not known. Murine embryonic mesenchymal stem cells, C3H10T1/2, were transduced with retrovirus encoding alpha-catenin (MSCV-alpha-catenin-HA-IRES-GFP). Cellular alpha-catenin expression was confirmed by western blot analysis using anti-HA antibody. In the presence of osteogenic media (10 mM beta-glycerol phosphate and 50 microgm/ml ascorbic acid) or Wnt-3A-conditioned medium, cells overexpressing alpha-catenin showed enhanced osteoblastic differentiation as measured by alkaline phosphatase (ALP) staining and ALP activity assay compared to the cells transduced with empty virus (MSCV-IRES-GFP). In addition, mRNA expression of osteocalcin and Runx2 was significantly increased compared to control cells. Cell aggregation assay revealed that alpha-catenin overexpression has significantly increased cell-cell aggregation. However, cellular beta-catenin levels (total, cytoplasmic-nuclear ratio) did not change by overexpression of alpha-catenin. Moreover, transient transfection of alpha-catenin did not increase TOPflash reporter acitivity nor was able to augment beta-catenin-mediated reporter activity. These results suggest that alpha-catenin overexpression increases osteoblast differentiation by increasing cell-cell adhesion rather than Wnt/beta-catenin signaling.

***Disclosures:* D. Kim**, *None.*

SA047

See Friday Plenary number F047.

SA048

Aging of Human Bone Marrow Stromal Cells: Role of WNT Pathways. L. Shen*, S. Zhou, J. Glowacki. Orthopedic Surgery, Brigham and Women's Hospital, Boston, MA, USA.

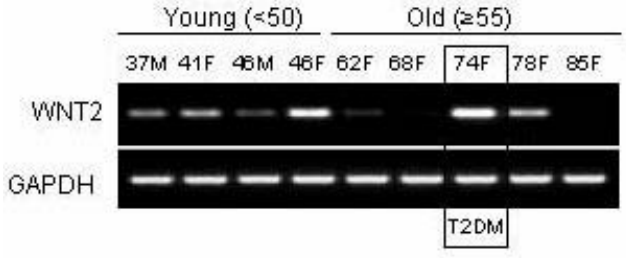
Human bone marrow stromal cells (hMSCs) include a fraction of osteoblast progenitors; we showed that there is an age-dependent decrease in their proliferation and osteoblast differentiation. In other cell types, WNT signaling maintains proliferation by stimulating cell division and inhibiting differentiation and apoptosis. Thus, we tested the hypothesis that WNT signaling may be related to aging in hMSCs. The first study tested whether there is an age related difference of WNT gene expression. The second study tested whether there is an effect of age on canonical WNT signaling in response to Parathyroid Hormone (PTH).

Bone marrow samples were obtained with IRB approval as femoral tissue discarded during total hip replacement for osteoarthritis. Low-density mononuclear cells were isolated by density centrifugation on Ficoll/Histopaque 1077(Sigma, MO). Adherent hMSCs were expanded 2-4 passages for experiments.

All Passage 2 (P2) samples of hMSCs from young subjects(<50y) expressed WNT2, a canonical WNT gene (Figure). In contrast, 3 of 4 samples from older subjects (>55y) showed no or low expression of WNT2. A notable additional sample (74 years old) with Type 2 diabetes mellitus (T2DM) as a comorbidity showed the highest expression of WNT2. All samples, including those with low or no WNT2, expressed strong signals for noncanonical WNT5a, and moderate signals for canonical WNT10b. A comparison of P2 with P4 cells showed differences attributable to in vitro effects: WNT 2 increased and WNT10b decreased with passage in many samples. These findings emphasize that passaging hMSCs may confound interpretation of expression profiling.

We assessed the effect of age on canonical Wnt signaling by measuring the effect of 10 nM PTH(1-34) on β-catenin at 6 h by Western immunoblot. In hMSCs from a 74-year-old subject, PTH did not activate β-catenin, compared with increased β-catenin (450%, 187%) in hMSCs from two younger subjects (34 and 42 years).

These pilot results indicate that 1) WNTs are expressed in hMSCs, 2) WNT2 expression is decreased with age, 3) *in vitro* passaging of cells may confound *in vivo* age differences, and 4) canonical WNT signaling in response to PTH was decreased with age. In conclusion, WNT2 may play a role in effects of aging on hMSCs.



***Disclosures:* L. Shen**, *None.*
This study received funding from: NIH-NIA.

SA049

Marked Induction of Endochondral Bone Formation by Overexpression of a Constitutively Active Gli2 in Mesenchymal Stem Cells Isolated from the Healing Site. Q. Wang*, C. Xie*, M. Xue*, X. Zhang. Orthopaedics, University of Rochester Medical Center, Rochester, NY, USA.

Hedgehog/Gli2 pathway has been critically implicated in endochondral bone formation during skeletal development. The hedgehog pathway is also activated during fracture repair, indicating a potential role of the pathway in bone repair process. Using a segmental bone graft transplantation model in mice, we have succeeded in isolation of a unique population of mesenchymal stem cells from periosteum at the early stage of healing. These cells express the typical mesenchymal stem cell markers Sca-I, SSEA4, CD105, CD29 and CD140 and demonstrated marked proliferation capacity *in vitro.* They are multipotent and capable of differentiating into osteoblasts, adipocytes and chondrocytes. Among growth factors and genes examined, we found that activation of Hedgehog signaling potently induced osteoblastic and chondrogenic differentiation of these cells *in vitro*. Furthermore, subcutaneous implantation of the isolated periosteal cells overexpressing a constitutively active Gli2 (ΔNGli2), the major transcriptional activator targeted by the hedgehog signaling pathway, led to marked and robust induction of endochondral bone formation *in vivo*. To further determine the mechanisms involved in Gli2 induced endochondral bone formation, we performed pellet culture using mesenchymal stem cells isolated from the healing site. We found that Gli2 overexpression increased Col2a1 and ColX gene expression by 25-fold and 18-fold respectively. Gli2 overexpression also markedly induced OSX gene expression by 5 fold and BMP-2 gene expression by 3 fold. To further determine whether chondrogenic and osteogenic differentiation induced by ΔNGli2 is mediated through BMP-2, we isolated mesenchymal stem cells from BMP-2 $^{f/f}$ mice. BMP-2 was eliminated by infection of an adenovirus expressing Cre recombinase. We found that elimination of BMP-2 blocked osteogenic differentiation induced by ShhN peptide and a hedgehog agonist Purmorphamine. However, BMP-2 elimination failed to block the osteogenic differentiation induced by ΔNGli2, suggesting additional mechanisms involved in Gli2 induced mesenchymal cell differentiation. Taken together our current data suggest that Hh/Gli2 may function as important signals for mesenchymal stem cell differentiation during repair and overexpression of Gli2 could be used in mesenchymal stem cell therapy for enhanced bone repair and regeneration.

***Disclosures:* X. Zhang**, *None.*
This study received funding from: NIH.

SA050

See Friday Plenary number F050.

SA051

Dermo1 Lineage Tracing Identifies Early Osteoprogenitor Cells in Adult Murine Bone Marrow Mesenchymal Stem Cell Cultures. P. Maye, Y. Liu*, L. Wang*, M. Kronenberg*, D. Rowe. Reconstructive Sciences, UCONN Health Center, Farmington, CT, USA.

Previous embryonic studies have shown that Twist 1 & 2 are expressed in early osteochondral progenitor cells and lie genetically upstream of Runx2 during osteogenesis. With this in mind, we hypothesized that Dermo1 (Twist 2) -Cre mice when crossed with Z/EG mice, a mouse line that ubiquitously expresses EGFP upon Cre Recombinase activity, may mark bone marrow derived osteoprogenitor cells and possibly multipotent mesenchymal stem cells in adult mice. While this approach is based on lineage tracing and results in a wide variety of mature cell types expressing EGFP, including bone cells and cartilage cells, the bone marrow compartment of F1 animals only contains a small cell population (~1%) that is EGFP positive. Preliminary attempts to FACS isolate this EGFP cell population directly from the bone marrow and test their osteogenic potential in vitro have proven difficult. We believe our current methodology for FACS isolation results in cell damage to the point where neither EGFP+ nor EGFP- cells attach to the culture dish. However, without FACS, standard bone marrow derived stromal cell cultures show that EGFP+ cells attach early in primary cultures. At day 3 of culture, EGFP positive cells can be observed during early colony formation. FACS isolation of EGFP positive cells from day 5 cultures reveals that these EGFP positive cells, which represent ~10% of the total cell population, when plated back into culture as a confluent spot retain very high osteogenic potential. These day 5 EGFP positive cells also have adipogenic potential and we currently are testing for their ability to differentiate into chondrocytes. To further confirm the osteogenic potential of this defined population in vivo, we carried out transplantation experiments where varying amounts of FACS isolated EGFP positive cells from day 5 stromal cell cultures were compared to EGFP negative cells and the matrix scaffold Helos alone in a critical size defect calvarial implant model. Quite strikingly, when 50,000 and 300,000 EGFP positive cells were loaded into the Helos scaffold, a dosage dependent increase in bone formation was observed in transplants 30 days later, while 100,000 EGFP negative cells or Helos alone resulted in no bone formation. These studies confirm that Dermo1-Cre x Z/EG leads to the identification of early osteoprogenitor cells and implicates an important role for twist genes in bone marrow derived mesenchymal stem cells.

***Disclosures:* P. Maye**, *None.*

SA052

In Bone, RPTPμ Is Exclusively Expressed in Osteocytes and May Affect Bone Mass. K. E. de Rooij[1], E. Waarsing*[2], E. de Wilt*[1], I. Que*[1], M. M. L. Deckers*[1], C. W. G. M. Löwik[1]. [1]Endocrinology, LUMC, Leiden, Netherlands, [2]Orthopaedics, Erasmus Medical Center, Rotterdam, Netherlands.

During bone formation, some osteoblasts are embedded within the newly formed matrix, where they differentiate into osteocytes. They develop long, slender cell processes, which they use to establish a three-dimensional cellular network within the mineralized matrix, analogous to the network formed by neuronal and endothelial cells. Their position within the mineralized matrix makes them very well-positioned to act as “the nerve cells of the bone”. It has now been generally accepted that osteocytes act as sensors of mechanical loading of the bone.
The receptor-like protein tyrosine phosphatase μ (RPTPμ) belongs a subfamily of transmembrane protein tyrosine phosphatases. RPTPμ is involved in cell-cell interactions and may play a role in the formation or maintenance of the cellular network. RPTPμ is expressed in cells in which cell-cell interactions are important, such as some types of neuronal cells, lung epithelium, cardiac muscle cells and endothelial cells. Strikingly, some of these cells also form a cellular network.
Recently, RPTPμ-knock out / LacZ knock-in mice have been described, in which the expression of the LacZ gene is controlled by the promoter of the RPTPμ gene. Long bones and calvariae of these transgenic mice were isolated and analyzed for ß-galactosidase activity by staining with X-gal. Histological analysis showed that only osteocytes exhibit ß-galactosidase activity. The blue staining was present throughout the cells and the cellular processes could easily be distinguished.
To investigate the possibility of generating osteocytes *in vitro*, bone marrow cells of the transgenic mice were cultured under osteogenic conditions. After 21-28 days, blue stained cells could be observed associated with nodules. Using an enzymatic LacZ assay we were able to quantify the amount of osteocytes formed. The addition of BMPs to the culture medium increased the number of LacZ positive cells. Histological analysis of these cultures revealed that all positive cells were surrounded by extracellular matrix and cellular processes could be observed. Thus, RPTPμ-knock out / LacZ knock in mice provide an excellent tool for the study of compounds on osteocyte differentiation *in vitro*.
To examine if the absence of RPTPμ gene had an effect on bone mass, we have isolated tibia of wild type and knockout mice (n=6) at 5 different age groups, up to 56 weeks for μCT analysis. Preliminary data of tibia from 44 weeks old mice indicate that lack of RPTPμ may result in lower bone mass. We conclude that RPTPμ could play a role osteocyte function. If absence of RPTPμ indeed results in lower bone mass, stimulating RPTPμ might increase bone mass. Therefore, RPTPμ may be a target for therapy in bone disorders.

Disclosures: ***K.E. de Rooij****, None.*

SA053

Endothelial Progenitor Cell Mobilization During Distraction Osteogenesis in Human. D. Lee*[1], T. Cho[2], H. Lee*[2], W. Yoo*[2], I. Choi*[2]. [1]Orthopaedic surgery, Kangwon National University, Chuncheon-city, Republic of Korea, [2]Orthopaedic Surgery, Seoul National University, Seoul, Republic of Korea.

Introduction: Distraction osteogenesis (DO) is a unique postnatal bone formation process which is characterized by profuse increment of vascularization. Recently osteoblast-lineage cells (OCs) and endothelial progenitor cells (EPCs) are reported to circulate in physiologically significant numbers and contribute to bone regeneration process. We investigated OC and EPC mobilization in patients undergoing limb lengthening.
Materials and Methods: Blood were drawn pre-operation, pre-distraction, and 2 weeks after the start of distraction in 25 patients undergoing limb lengthening. Peripheral blood mononuclear cells (PBMNCs) were isolated and cultured under endothelial cell growth medium. After 2 weeks, the number of EPC colonies which are stained with 1,1-dioctadecyl-3,3,3,3-tetramethylindocarbocyanine-conjugated acetylated-low density protein and fluorescein isothiocyanate-labeled lectin was counted. In addition, we performed fluorescence-activated cell sorting (FACS) analysis of freshly isolated PBMNCs using antibodies to bone-specific alkaline phosphatase (ALP) to identify circulating OCs and antibodies to CD34 and vasculoendothelial growth factor receptor 2 (VEGFR2) to identify circulating EPCs.
Results: The number of EPC colonies significantly increased during distraction period. FACS analysis demonstrated that the frequency of VEGFR2 (+) cells and CD34 (+) cells, which are EPC-enriched fractions, significantly increased post-distraction while there were no differences in AC133 (+) and ALP (+) positive cells. The number of EPC colonies was correlated with the proportion of circulating CD34 (+) cells in PBMNCs.
Conclusions: EPCs are mobilized during distraction osteogenesis by distraction strain while OCs are not. These data suggest that EPC mobilization enhanced by distraction strain contributes to increased vascularization during DO, but bone regeneration process during DO is not associated with systemic OC mobilization.

Disclosures: ***D. Lee****, None.*
This study received funding from: Seoul National University Hospital Research pund.

SA054

See Friday Plenary number F054.

SA055

Widespread Expression of Sclerostin in Adult and Embryonic Cartilage and Bone, Cells of the Developing Central Nervous System and Epithelia of the Embryonic Kidney and Intestine. S. Sommer*[1], J. P. Grande*[2], R. Kumar[1]. [1]Nephrology Research, Mayo Clinic, Rochester, MN, USA, [2]Anatomic Pathology, Mayo Clinic, Rochester, MN, USA.

Sclerostin, an inhibitor of bone formation, is believed to be produced exclusively by osteocytes in adult tissues. We used a specific polyclonal antibody to examine expression of sclerostin in adult and embryonic mouse tissues. As previously reported, in adult bone, sclerostin immunostaining was detected in osteocytes and in the canalicular network of Haversian systems. We now show that at the growth plate, sclerostin immunostaining is present in dividing chondrocytes and osteoblasts of adult mouse bone. Less intense staining is seen in mature chondrocytes. Adult liver, spleen, and kidney exhibit no staining. Immunostaining with pre-immune serum is negative.
In the developing skeleton, sclerostin staining is noted in vertebrae and ribs, specifically in developing chondrocytes. Mature chondrocytes do not stain for sclerostin. Osteoblasts stain for sclerostin in the developing vertebral column. Similar patterns of staining are noted in ossification centers at the base of the skull. Immunostaining with pre-immune serum is negative.
Sclerostin immunostaining is noted in cells of the developing neural crest starting at day 8 p.c. Sclerostin immunostaining is observed in the developing dorsal root ganglia beginning at day 12 p.c. Sclerostin staining remains prominent in all developing bone tissues through the entire growth of the embryonic mouse. Sclerostin immunostaining is observed in scattered epithelial cells of the developing intestine and kidney starting at day 11 and disappearing by day 16 p.c. The sclerostin immunostaining in the kidney is most prominnent in epithelial cells of the S-shaped tubules and epithelial cells of the intestine. No expression of sclerostin is noted in the developing heart, liver, muscle, or skin.
Conclusion. In adult bone, sclerostin is expressed not just in osteocytes but also in dividing chondrocytes and in osteoblasts found in the growth plate suggesting a function for sclerostin in the latter cells. In addition, sclerostin is prominently expressed in chondrocytes and osteoblasts of the developing mouse skeleton. Sclerostin is also expressed in the epithelium of the intestine and kidney suggesting an importance not only to bone development but also in epithelial development.

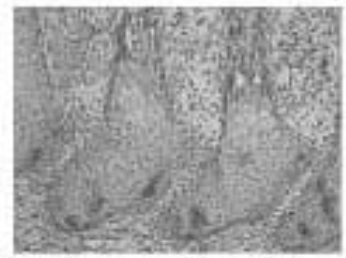


Fig 1. Sclerostin in developing vertebral column, 15 day p.c. embryo. 200 X.

Disclosures: ***S. Sommer****, None.*
This study received funding from: NIH.

SA056

Osteocytes can Modulate Osteoblastic Activity via NPY Signaling. J. C. Igwe*, S. M. San Miguel*, F. Paic*, H. T. Li*, I. Kalajzic. Dept. of Reconstructive Sciences, Uni. of Conn. Health Center, Farmington, CT, USA.

Osteocytes are characterized by long neuronal-like cell processes, which may actively participate in the release of molecules that modulate the function of other surrounding bone cells. The location of osteocytes and their inherent inability to divide represents a major obstacle for studying osteocyte biology. We have previously shown that dentin matrix protein 1 (DMP1) is preferentially expressed in cells embedded within the bone matrix (osteocytes) or in partially embedded cells (preosteocytes). This was the key observation leading to generation of an osteocyte-specific GFP transgenic mouse (DMP-1GFP). To investigate the expression of regulatory molecules produced by osteocytes, we utilized dual transgenic mouse in which osteocytes are idenitified by DMP-1-GFP(green), while osteoblasts are labeled by Col2.3GFP(blue). An in vivo gene expression analysis was generated by fluorescence activated sorting of neonatal calvarial cells derived from these dual transgenic mice. It was an intriguing observation that the osteocytes expressed neuropeptide Y (NPY), a gene whose role in the central control of bone mass has been well established. We confirmed this result by immunohistochemical detection of NPY in osteocytes. Furthermore, NPY mRNA expression was observed in DMP1GFP+ cells (osteocytes) and Col2.3GFP+ cells (osteoblasts/osteocytes) derived from fluorescent sorting of primary calvarial osteogenic cultures.
RNA analysis of the isolated osteocytes demonstrated increased levels of NPY mRNA transcripts in both DMP1GFP+ and Col2.3GFP+ populations. In addition, we investigated

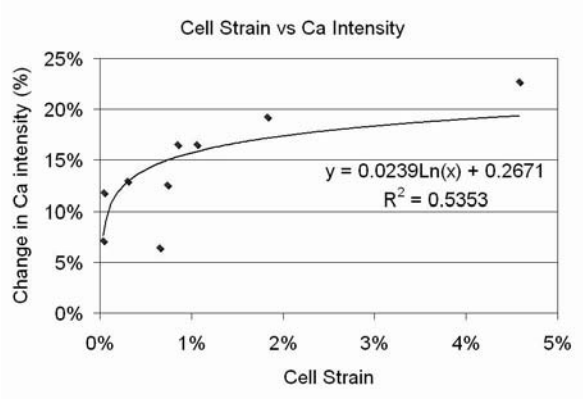
the expression of NPY receptors in cultured osteoblast lineage cells. qPCR analysis of cultured calvarial osteoblasts demonstrated that mRNA of the NPY receptor subtypes Y1 and Y6 increased significantly between day 7 and day 14 of culture. We further investigated the effect of NPY at this stage of culture by in vitro treatment with NPY protein. Northern blot analysis of RNA from 1 nM NPY treated calvarial osteoblasts, showed a significant reduction of osteocalcin and bone sialoprotein expression at day 14 of culture. These results demonstrate the effect of NPY on mature osteoblasts, suggesting a novel, local regulation of osteoblast function by osteocytes via NPY signaling.

Disclosures: ***J.C. Igwe****, None.*
This study received funding from: NIH DE016495-02.

SA057

Direct Correlation of Osteocyte Deformation with Calcium Influx in Response to Fluid Flow Shear Stress. A. R. Bonivtch[1], L. F. Bonewald[2], J. Ling*[1], J. X. Jiang[3], D. P. Nicolella[1]. [1]Southwest Research Institute, San Antonio, TX, USA, [2]University of Missouri at Kansas City School of Dentistry, Kansas City, MO, USA, [3]University of Texas Health Science Center, San Antonio, TX, USA.

Osteocytes are generally accepted to be the mechanosensors of bone regulating both resorption and formation. Calcium signaling plays an integral role in osteocyte signaling of bone formation. These cells are highly sensitive to fluid flow derived shear stress thought to occur as bone fluid passes through the osteocyte lacuno-canalicular network. However, the resulting individual cell strain has yet to be quantified and associated with any specific individual cell biological response. We have shown previously a correlation of deformation with prostaglandin production for a population of MLO-Y4 osteocyte like cells. The purpose of this study was to directly measure individual osteocyte deformation and intracellular calcium levels in response to fluid flow. The resulting deformation of each cell was then correlated to its individual biological response.
Fluo-4, AM-loaded osteocyte-like MLO-Y4 cells were exposed to uniform laminar fluid flow of 8 dynes/cm^3 in a closed system. The upregulation of intracellular calcium was determined from the difference in fluorescence intensity from the cell's basal level prior to exposure to fluid flow and the level immediately following the application of fluid flow. The resulting deformation of each individual osteocyte was determined using digital image correlation.
The average strain experienced by the cells was 1.12% with a standard deviation of 0.01%. The maximum and minimum strains were 4.58% and 0.03% respectively. The average upregulation of intracellular calcium in the cells was found to be 13.99% with a standard deviation of 0.05%. The maximum and minimum increases in intracellular calcium were 22.71% and 6.45% respectively. Moreover, the MLO-Y4 cells showed an increase in intracellular calcium concentration with an increase in cell strain in response to an applied fluid flow of 8 dynes/cm^3 (Figure 1). Osteocytes exposed to a uniform fluid flow stimulus experienced a range of strains and individual cell deformations correlated in a dose dependent manner to increases in intracellular calcium concentrations. As mechanosensing and signaling occurs at the single osteocyte level, it is important to understand how cell deformation is translated in a wide range of osteocyte responses.



Disclosures: ***A.R. Bonivtch****, None.*
This study received funding from: NIH.

SA058

See Friday Plenary number F058.

SA059

Expression of Human Sclerostin In Heterologous Eukaryotic Insect and *E. coli* Expression Systems. T. A. Craig*, Z. Ryan*, R. Kumar. Nephrology Research, Mayo Clinic, Rochester, MN, USA.

Sclerostin is an ~22 kDa secreted protein expressed by osteocytes which inhibits bone formation. Several sclerostin gene mutations result in a general progressive overgrowth and sclerosis of the skeleton. Sclerostin antagonizes canonical Wnt signaling pathways that control bone formation by binding to Wnt co-receptor protein, low-density lipoprotein receptor-related protein 5 and 6 (LRP5/6). High bone mass in sclerosteosis and Van Buchem disease may thus result from increased Wnt signaling due to the reduced sclerostin expression. Sclerostin mRNA has been detected in aortic tissue, in the otic vesicle and in odontoblasts suggesting that sclerostin may have functions other than bone density regulation.
In order to examine biochemical and biophysical properties of sclerostin, and its interaction with other signaling molecules, we have employed several different bacterial and insect expression systems in order to obtain soluble sclerostin. The full length cDNA for the secreted form of human sclerostin, amino acids 24-213, was expressed in the pET 28a(+) and pET 42a(+) *E. coli* expression vectors as a soluble protein with amino-terminal V5 and 6xHis tags. When sclerostin was expressed in *E. coli* using the pET 28a(+) vector nickel affinity chromatography was used for initial purification. The protein expressed in *E. coli* with the pET 42a(+) vector was a fusion protein with glutathione S-transferase (GST) and was purified using glutathione sepharose. The expression of sclerostin as a fusion protein with maltose binding protein (MBP) into the bacterial periplasmic space of BL21Star *E. coli* with the vector, pMAL-p4E, yielded, on osmotic shock of cells, some full-length 24-213 sclerostin-MBP and large amounts of MBP alone.
We used two insect expression systems for expression of sclerostin. *Trichoplusia ni* (High Five) insect cells were stably transformed with a pIB/V5-His-melittin secretory signal-human 24-213 sclerostin construct. Transfected cells were selected with Blasticidin S. Secreted sclerostin was detected in the cell medium by Western blotting with anti-V5 horseradish peroxidase antibody. The cDNA for the secreted form of human sclerostin, amino acids 24-213, was also ligated in to the pTriEx-5 insect secretion vector. TriEx™ Sf9 *Spodoptera frugiperda* insect cells using GeneJuice® transfection reagent were transiently transfected in order to express sclerostin as a secreted protein.
Conclusion: Soluble sclerostin can be expressed in *E. coli* as a histidine or GST-fusion protein. Secreted, soluble sclerostin is readily expressed in insect cells using permanent and transient expression methods.

Disclosures: ***T.A. Craig****, None.*

SA060

Mechanical Perturbation of Integrin a5 with or without Association with Fibronectin Opens Connexin 43-Hemichannels in Osteocytes - a Mechanism for Release of Small Signaling Molecules in Response to Loading. S. Burra[1], A. J. Siller-Jackson*[1], M. A. Harris*[2], S. E. Harris[2], G. Weber*[3], D. DeSimone*[3], L. F. Bonewald[4], E. Sprague*[5], M. A. Schwartz*[6], J. X. Jiang[1]. [1]Biochemistry, University of Texas Health Science Center, San Antonio, TX, USA, [2]Periodontics, University of Texas Health Science Center, San Antonio, TX, USA, [3]Cell Biology, University of Virginia, Charlottesville, VA, USA, [4]Oral Biology, University of Missouri, School of Dentistry, Kansas City, MO, USA, [5]Radiology, University of Texas Health Science Center, San Antonio, TX, USA, [6]Microbiology, Biomedical Engineering and Cardiovascular Research, University of Virginia, Charlottesville, VA, USA.

Connexin 43 hemichannels are un-opposed halves of gap junctions that play a role in osteocyte viability and signaling. Mechanical stimulation in the form of fluid flow shear stress has been shown to induce the opening of hemichannels in MLO-Y4 osteocyte-like cells releasing prostaglandins, ATP and potentially other signaling molecules important for bone formation. In a search for potential mechanosensors associated with hemichannels, Cx43 was found to consistently co-localize with the α5 and β1 integrin complex on the cell but not found to co-localize with the focal adhesion proteins, vinculin or paxillin. Co-immunoprecipitation experiments validated this association between Cx43 and α5/β1 integrin.
To determine whether Cx43/integrin α5 interactions play a role in the opening of hemichannels in response to mechanical stimulation, the MLO-Y4 cell model was used. Blocking antibody to α5 integrin inhibited and α5 integrin siRNA totally abolished the opening of hemichannels and associated prostaglandin release in response to shear stress. No changes were observed in cell surface expression of Cx43. Magnetic beads coated with either anti-α5 integrin antibody or fibronectin were perturbed by a magnetic field after attachment to the cell surface. This resulted in the opening of hemichannels as visualized by dye uptake. In contrast, control beads coated with poly-lysine or anti-CD44 antibody failed to induce the opening of hemichannels when moved at the same magnitude. Similar to non-treated control, extracellular matrix substrate for α5 integrin, fibronectin, fibronectin-conjugated beads or integrin binding ligand RGD peptide did not have any effect on the opening of hemichannels by shear stress, indicating that α5 integrin, not fibronectin, is the target for opening hemichannels by shear stress. Together, these results suggest that integrin α5/β1 may serve as a mechanical tether or tensor mediating the effects of shear stress in regulating the opening of Cx43-forming hemichannels and that this *novel* function of integrin can be independent of its association with extracellular matrix.

Disclosures: ***J.X. Jiang****, None.*
This study received funding from: National Institute of Health.

SA061

Proteoliposomes Carrying Alkaline Phosphatase and Nucleotide Pyrophosphatase/ Phosphodiesterase-1 as Matrix Vesicle Mimetics. P. Ciancaglini*[1], A. M. S. Simao*[1], M. C. Yadav[2], S. Narisawa*[2], M. F. Hoylaerts*[3], J. L. Millan[2]. [1]Departamento de Quimica, FFCLRP-USP, Ribeirao Preto, Brazil, [2]Sanford Children's Health Research Center, Burnham Institute for Medical Research, La Jolla, CA, USA, [3]Center for Molecular and Vascular Biology, University of Leuven-Campus Gasthuisberg, Leuven, Belgium.

Experimental evidence indicates that endochondral calcification is mediated by chondroblast- and osteoblast-derived matrix vesicles (MV). The primary function of tissue-nonspecific alkaline phosphatase (TNAP) is to degrade extracellular inorganic pyrophosphate (ePPi), a potent mineralization inhibitor, which is produced ectoplasmically by the enzymatic activity of nucleotide pyrophosphatase/ phosphodiesterase-1 (NPP1), restricting the concentration of ePPi, to maintain a Pi/PPi ratio permissive for normal bone mineralization. Both these enzymes act at the level of the membrane in osteoblasts and in osteoblast-derived MVs. In this study we have used a liposome system to reconstitute proteoliposomes harboring TNAP alone, NPP1 alone, or both in combination, to study the kinetic behavior of these enzymes in a lipophilic membrane environment. TNAP and NPP1 were expressed in CHO cells, solubilized with polidocanol and inserted into dipalmitoylphosphatidylcholine (DPPC) liposomes as previously standardized1. Polidocanol-solubilized detergent-free TNAP and NPP1, alone or in combination showed the ability to anchor to DPPC liposomes. The enzyme activities were measured at pH 7.4, for the substrates ATP, ADP, AMP and PPi, by measuring the amount of inorganic phosphate liberated as a function of time, as previously described1. Hydrolyzed ATP nucleotide intermediates were separated and quantitated by HPLC. NPP1-liposomes exclusively hydrolyzed ATP into AMP and PPi. In contrast, TNAP-liposomes, as well as TNAP+NPP1-liposomes hydrolyzed ATP, ADP, AMP and PPi. Hence, the simultaneous presence of TNAP and NPP1 on a proteoliposome membrane enables the complementary hydrolysis of natural phospho-substrates and their intermediates, leading to very efficient phosphate accumulation in the immediate microenvironment of the phospholipid membrane surface. Therefore, we conclude that the controlled reconstitution of MV-associated phosphatases and phosphodiesterases in proteoliposome membranes generates the correct microenvironment to study phospho-substrate catalysis and PPi degradation, allowing more detailed studies of those catalytic processes that initiate skeletal calcification.
Financial Supports: FAPESP, CNPq, CAPES and DE12889 and AR47908 from NIH.
1Camolezi et al., Construction of an alkaline phosphatase-liposome system: a tool for biomineralization study. Int. J. Biochem. Cell Biol. 34: 1091-1101 (2002).

***Disclosures:* *A.M.S. Simao**, None.*
This study received funding from: NIH.

SA062

See Friday Plenary number F062.

SA063

Molecular Mechanisms Underlying Matrix Vesicle-induced Mineralization During Bone Formation: An Enzyme Kinetic Approach. P. Ciancaglini*[1], A. M. S. Simao*[1], M. C. Yadav[2], S. Narisawa*[2], C. Farquharson[3], M. F. Hoylaerts*[4], J. L. Millan[2]. [1]Departamento de Quimica, FFCLRP-USP, Ribeirao Preto, Brazil, [2]Sanford Children's Health Research Center, Burnham Institute for Medical Research, La Jolla, CA, USA, [3]Bone Biology Group, Roslin Institute, University of Edinburgh, Edinburgh, United Kingdom, [4]Center for Molecular and Vascular Biology, University of Leuven-Campus Gasthuisberg, Leuven, Belgium.

Mineralization of cartilage and bone occurs by physico-chemical and biochemical processes that together facilitate the deposition of hydroxyapatite in specific areas of the extracellular matrix, mediated by chondroblast- and osteoblast-derived matrix vesicles (MVs). The primary function of tissue-nonspecific alkaline phosphatase (TNAP) is to degrade extracellular inorganic pyrophosphate (ePPi), a mineralization inhibitor, which is produced by nucleotide pyrophosphatase/phosphodiesterase-1 (NPP1), restricting the concentration of ePPi, to maintain a Pi/PPi ratio permissive for normal bone mineralization. We studied the efficiency of phospho-substrates catalysis by osteoblast-derived MVs, kinetically analyzing the hydrolysis of ATP, ADP and PPi by isolated native wild-type (WT), PHOSPHO1 null, TNAP null and NPP1 null MVs. The enzyme activities were determined at pH 7.4 by measuring the amount of inorganic phosphate liberated as a function of time, as previously described1. Throughout catalysis, reaction nucleotide intermediates, during hydrolysis of ATP and ADP were monitored via separation and quantitation in HPLC. Comparison of the catalytic efficiencies measured for TNAP-/-, NPP1-/- and PHOSPHO1-/- MVs to those of native MVs identified ATP as the main substrate, hydrolyzed by WT MVs. Hydrolysis of ATP was significantly reduced in the absence of TNAP, but also of PHOSPHO1, implicating both enzymes in the processing of ATP by MVs. Also, we observed deficient PPi and ADP hydrolysis in TNAP-deficient MVs underscoring that, unlike for the hydrolysis of ATP, PPi is primarily processed by TNAP. The lack of NPP1 did not significantly affect the kinetic parameters of hydrolysis when compared to WT MVs, for any of the substrates. We conclude that the hydrolysis of phospho-substrates in MV membranes results from the concerted action of phospholipid-dependent NPP1, TNAP and PHOSPHO1, enabling hydroxyapatite mineralization under conditions of restricted PPi accumulation.

Financial Supports: FAPESP, CNPq, CAPES and DE12889, AR47908 and AR53102 from NIH.
1Camolezi et al., Construction of an alkaline phosphatase-liposome system: a tool for biomineralization study. Int. J. Biochem. Cell Biol. 34: 1091-1101 (2002).

***Disclosures:* *M.C. Yadav**, None.*
This study received funding from: NIH.

SA064

MEPE Expression Is Regulated by BMP-2 Signaling Through the Activation of Its Downstream Transcription Factors, Dlx3, Dlx5 and Runx2. Y. Cho, J. Park*, W. Yoon*, K. Woo*, J. Baek*, H. Ryoo*. Department of Cell and Developmental Biology, School of Dentistry and Dental Research Institute, Seoul National University, Seoul, Republic of Korea.

MEPE (Matrix Extracellular PhosphoglycoprotEin), also called OF45 (osteoblast/ osteocyte factor 45), is expressed in osteoblasts, osteocytes and odontoblasts during mineralization. MEPE is a 525-amino acids extracellular matrix protein, and has osteogenic activity through its centrally located GAG- and cell-attachment motif, AC-100. On the contrary, it has mineralization inhibitory activity through its C-terminal ASARM motif (minhibin). Recent reports indicated that MEPE is a standard marker of osteocytes, however, it still remained unclear how its expression is regulated by extracellular signals. In this study, we tried to illuminate upstream signals that are regulating MEPE expression. Our previously cDNA microarray analysis indicated that mRNA level of MEPE is about 27.9 folds higher in mineralizing mouse calvarial tissue than in unmineralized suture tissue. Long-term culture of MC3T3-E1 cells in osteogenic medium showed that MEPE expression is stimulated 200,000 folds at 21 day of culture (compared to 1 day). These results implicated that MEPE expression is a mineralizing tissue-specific, especially at the late mineralization stage. Our previous reports have shown that BMP-signaling is utmost important for the mineralization of the calvarial bones. In addition, its downstream transcription factors such as Dlx3, Dlx5 and Runx2 are very important modulators of calvarial bone mineralization. Thus we have tested BMP-2 effect on MEPE expression in osteogenic cells. BMP-2 (100 ng/ml) treatment to MC3T3-E1 cells strongly increased MEPE mRNA level by 25 folds. Similarly, the overexpression of BMP-2 downstream transcription factors such as Dlx3, Dlx5, and Runx2 also up-regulated MEPE mRNA expression as well as MEPE promoter activity. Moreover, co-transfection of Dlx5 and Runx2 with MEPE reporter vector showed that there was a mild synergistic effect on the promoter activation. In contrast, knock-down of Smad1/5, Dlx3/5 or Runx2 by their siRNA treatment downregulated MEPE mRNA level even in the presence of BMP-2. With a serial MEPE promoter deletion analysis, site-directed mutagenesis experiments and electrophoretic gel mobility shift assays, we specified a couple of homeodomain and Runx2 response elements, respectively, in the proximal promoter of MEPE. Taken together, MEPE expression is specific in the late mineralization stage of in vitro cell culture, and is strongly stimulated by BMP-2 signaling through the activation of its downstream transcription factors, Dlx3, Dlx5 and Runx2.

***Disclosures:* *Y. Cho**, None.*
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SA065

TIEG1 KO Mice Display Defects in the Bone Matrix Immediately Surrounding Osteocytes. O. Haddad[1], J. R. Hawse[2], M. Subramaniam[2], C. Pichon[3], T. C. Spelsberg[2], S. F. Bensamoun[1]. [1]UMR CNRS 6600, Biomecanique et Bioingenierie, Universite de Technologie de Compiegne, Compiegne, France, [2]Department of Biochemistry and Molecular Biology, Mayo Clinic College of Medicine, Rochester, MN, USA, [3]Cnrs upr 430, Centre de Biophysique Moleculaire, Orleans, France.

Osteocytes constitute a three-dimensional network in bone and play crucial roles in cell-cell communication and mechanotransduction. Osteocytes communicate with each other, and with cells on the bone surface, via long cytoplasmic extensions referred to as canaliculi. Through the development of TGF beta Inducible Early Gene 1 (TIEG1) KO mice, we have demonstrated that TIEG1 plays an important role in osteoblast-mediated bone mineralization [1], in bone thickness and resistance to mechanical strain as well as in osteocyte density in cortical bone [2]. In order to further examine the osteocyte phenotype observed in these animals, four femurs were extracted from female TIEG1 KO and wild-type mice. Transverse cross sections were cut in the middle of the diaphysis and sections were placed in a fixative and decalcifying solution. Thin femoral cross sections (5µm) were prepared from these samples for transmission electron microscopy. Twenty osteocytes from each TIEG1 KO and wild-type (WT) femur were observed for differences in cell morphology and the structure of the bone matrix surrounding each cell. This analysis revealed differences in the unmineralized matrix surrounding osteocytes in TIEG1 KO mice relative to wild-type controls. Figure 1 highlights the unmineralized matrix (doted line) immediately surrounding individual osteocytes. A ratio between the cell surface and the unmineralized matrix was calculated and the results indicate that significantly less (2 fold) unmineralized matrix surrounds TIEG1 KO osteocytes relative to WT controls. Currently, little is known about the role and functions of the bone matrix immediately surrounding osteocytes. However, the present study clearly reveals differences in this bone micro-environment in TIEG1 KO mice and suggests that the bone remodeling and mechanosensing properties of these cells may be compromised in the absence of TIEG1 expression. Further investigations will be performed on cellular and molecular levels to determine presicely the impact of TIEG gene invalidation on

osteocytes biology.
[1] M. Subramaniam, et al. Mol Cell Biol 2005, 1191-1199
[2] S.F. Bensamoun, et al. Bone 2006, 39, 1244-1251

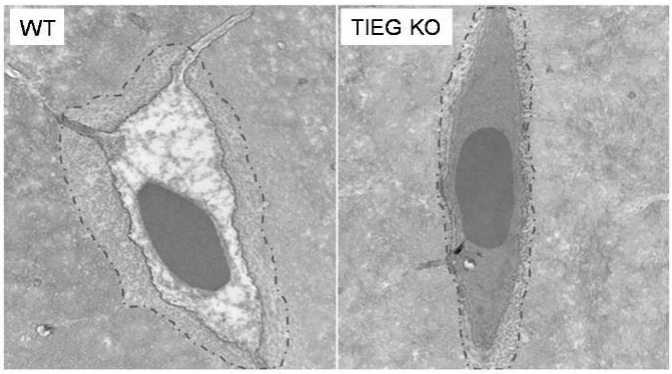


Figure 1: Electron micrographs depicting a reduced unmineralized matrix area in TIEG1 KO mice. Representative transmission electron microscopy images showing osteocytes in the cortical region of the wild-type (WT) and TIEG1 KO mouse femurs (TIEG KO). Unmineralized matrix areas are repsented with doted lines.

Disclosures: ***O. Haddad****, None.*

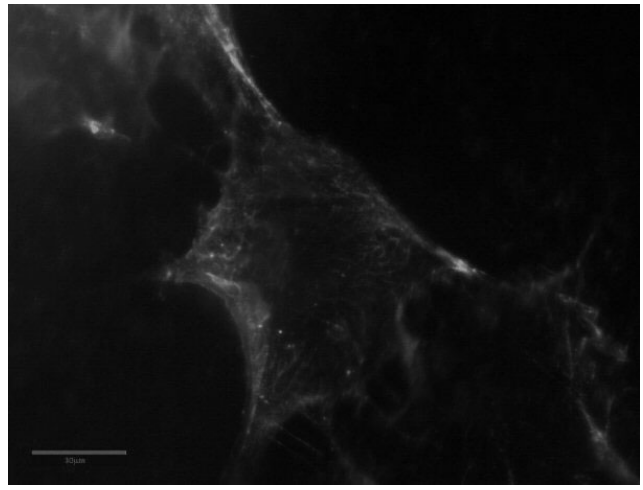
SA066

See Friday Plenary number F066.

SA067

The Response of Matrix Synthesizing MLO-A5 Cells to Fluid Shear. H. L. Morris*[1], A. Sittichokechaiwut*[1], C. R. Jacobs*[2], J. W. Haycock*[1], G. C. Reilly[1]. [1]Engineering Materials, University of Sheffield, UK, Sheffield, United Kingdom, [2]Mechanical Engineering, Stanford University, Stanford, CA, USA.

Bone remodels dependent on the mechanical loading it receives. For this to occur bone must be able to sense mechanical load in order to effect changes in the tissue architecture. Osteocytes are believed to be the cell type within bone which are sensitive to mechanical stimulation [1]. The interstitial fluid around osteocytes has been shown to be displaced by bone strain. Mechanosensitivity is proposed to result from the application of shear forces from fluid movement on the osteocytes. In tissue engineered bone, osteoblasts and their precursors must directly sense and respond to mechanical forces in a less constrained environment. The aim of the current work is to investigate responses to fluid flow in the cell line MLO-A5. These cells actively and rapidly synthesize bone matrix and have been reported to represent transitional cells between osteoblasts and osteocytes, also termed 'osteoid osteocytes' [2].
Intracellular calcium release in response to fluid flow was examined using the calcium indicator fluo-4. MLO-A5 cells were grown for three days in static culture on the base of a closed parallel plate flow chamber (Ibidi Integrated BioDiagnostics, GER), cells were then loaded with the indicator and subjected to pulsatile fluid flow (0.3Pa). A transitionary increase in intracellular calcium was observed a few seconds after commencement of flow. Gene expression of Collagen I and Osteopontin was analysed using RT-PCR and was seen to be upregulated two fold, 24 hours after cells were subjected to a flow of 2 Pa for 2 hours. To investigate the effects of flow on the longer term response of matrix production cells were stimulated by flow for 2 hours (2Pa) on day 3 of culture and collected on day 8. An increase in collagen production was observed in cells subjected to flow compared to no flow controls as assessed by sirius red assay.
Immunofluorescence showed that MLO-A5 cells possess both a hyaluronan (HA)-rich glycocalyx and primary cilia both putative mechanosensors of fluid shear in other bone cell types. In conclusion, we have established MLO-A5s will be a good model cell type with which to study the fluid flow responses of matrix synthesising osteoblasts.
Image showing HA glycocalyx of MLO-A5 cells, stained with HABP-FITC.



1. L. F. Bonewald. Bone 42(2008) 606-615
2. Y. Kato. J Bone Miner Res. 16(9)(2001) 1622-33

Disclosures: ***H.L. Morris****, None.*
This study received funding from: EPSRC White Rose Doctorial Training Centre.

SA068

Notch Signaling Regulates Osteoblast Maturation Transition to Mineralization Phase. S. Gao*, J. Hock, P. Liu. Maine Institute for Human Genetics and Health, Brewer, ME, USA.

Notch signaling pathway is believed to control binary stem cell-fates in development. However, its precise physiological role, and temporal and spatial expression in postnatal skeletal development remains largely unknown. To address this issue, we utilized Notch GFP reporter mouse and conditionally inducible gain or loss of Notch function mouse models. To locate GFP expression as a marker for Notch activity, we used fluorescent microscopy to examine frozen bone sections from Notch GFP mice (including long bones, spines, calvarias and tails in young and adult, both sex). GFP positive cells were present on the surface of trabecular bone and within mineralized matrix. GFP expression was greatly increased in areas of high bone formation activity in the primary spongiosa underlying growth plate in young mice. To determine if this Notch expression was restricted to osteoblast lineage, mouse bone marrow stromal cells (mBMSC) from GFP mice were isolated, and induced to differentiate with osteogenic media into mature Ob. GFP expression was monitored throughout of the 21 days culture period. Although no GFP was detectable in the early stage of culture (before day 7), surprisingly strong GFP expression was observed specifically in cells at the time mineralization initiated; this expression disappeared when mineralization completed. To gain functional insight of the Notch activity in Ob lineage progression, we cultured mBMSCs from mice either with conditionally ectopic expression of active intracellular Notch domain (NICD) or mice in which Notch ligand-Jagged 1(JAG1cKO) was knocked out. Cre was delivered to the cultures by adenovectors. Mineralization increased with increased NICD activity, but failed to occur in JAG1cKO. In the latter case, cells retained fibroblast-like morphology, and did not enter osteogenic differentiation. Collectively, these data provide new insights into the role of Notch in bone, and suggest the novel hypothesis that Notch pathway is critical in regulating progression into the mineralizing phase of Ob maturation.

Disclosures: ***S. Gao****, None.*
This study received funding from: Maine Institute for Human Genetics.

SA069

See Friday Plenary number F069.

SA070

Enrichment of Type XI Collagen and 6b N-Terminal Domain at Sites of Mineral Nucleation Within Osteoblastic Cultures. N. T. Huffman*[1], C. Chaoying*[2], S. V. Chittur*[3], J. T. Oxford[4], J. A. Keightley*[1], R. J. Midura[5], J. P. Gorski[1]. [1]Univ. of Missouri-Kansas City, Kansas City, MO, USA, [2]Tibet Univ. Med. College, Lhasa, China, [3]Univ. at Albany, Rensselaer, NY, USA, [4]Boise State Univ., Boise, ID, USA, [5]Cleveland Clinic, Cleveland, OH, USA.

Alternatively spliced 6b and 8 isoforms of type XI collagen are known to be associated with the process of embryonic bone formation. Type XI and I collagens co-exist in fibrils in bone and form hetero-dimeric complexes *in vitro* upon which type I fibrils can be built. Our goal was to determine when and where type XI RNA and protein were expressed during mineralization of UMR 106-01 osteoblastic cultures; nucleation of mineral occurs within extracellular biomineralization foci (BMF) in this model. Some cultures were treated with protease inhibitor AEBSF which blocks mineralization and activation of PCOLCE, an enhancer of BMP-1 processing of collagen. Type XI collagen expression increased 1.7-fold between 40 and 64 h after plating, just prior to when BMF are competent to mineralize. During this same period collagen I synthesis did not change suggesting that type XI may exhibit functions distinct from a role in type I fibrillogenesis. Cell layer fractions were also extracted sequentially with EDTA and then with 8 M urea/2% CHAPS; cell layer and media fractions were then subjected to western blotting. A 60 kDa N-terminal domain (NTD) was identified in EDTA and urea extracts using an antibody recognizing all type XI splice variants. NTDs were only detected in the media fraction in the absence of ß-glycerol phosphate or with AEBSF. After AEBSF treatment, urea extracts contained elevated amounts of 60 kDa NTD. Both 6b and 8 splice variant forms were found to be expressed by UMR cells as 60 kDa and 110 kDa bands, respectively. Identification of alpha 1 chain 8 epitope was confirmed by mass spectral peptide mapping. However, neither 6b nor 8 isoforms were detected in the urea extract suggesting the 60 kDa NTD in AEBSF-treated cultures is composed of the 6a alternative splice form, which is not associated with mineralization. Finally, a 60 kDa NTD reactive with 6b splice variant specific antibodies was enriched preferentially in mineralized BMF, whereas the 8 splice form is not. These results indicate a clear distinction between the localization and distribution of up to three different alternatively spliced isoforms of type XI collagen. Based on extraction with EDTA and on direct isolation by laser capture microscopy, the 6b isoform NTD is enriched in extracellular BMF. When mineralization is blocked by AEBSF, NTDs apparently containing the 6a epitope become more tightly bound to the cell layer. The enrichment of a large NTD expressing 6b epitope only within BMF, sites of initial mineral nucleation, suggests a functional or structural role in this process.

Disclosures: ***J.P. Gorski****, BoneMetrics LLC 5.*
This study received funding from: NIH AR052775 (JPG).

SA071

Lithium Affects Matrix Mineralization by Decreasing Tissue Non-specific Alkaline Phosphatase Levels in Osteoblasts. J. Li*, M. Murshed. McGill University, Montreal, QC, Canada.

Mineralization of bone extracellular matrix is achieved, at least in part, by the unique co-expression in osteoblasts of several broadly expressed genes. One of these genes *alkaline phosphatase, liver/bone/kidney* (*Alpl*) encodes a cell membrane-bound phosphatase, which cleaves inorganic pyrophosphate, a ubiquitously present mineralization inhibitor. We recently observed that addition of lithium ions to the cell culture medium prevented *in vitro* mineral deposition by reducing the alkaline phosphatase enzymatic activity in mouse primary osteoblasts. This reduced activity was not due to a direct inhibition of the enzyme by lithium ions as it was the case with some other magnesium-dependent phosphatases. Also, lithium treatment did not increase the release of the cell membrane-bound enzyme into the culture medium. Interestingly, we observed a decrease in alkaline phosphatase protein levels in the lithium treated cells. When added to the culture medium, recombinant bone morphogenetic protein-2 (BMP-2) reversed the effects of lithium on osteoblasts, suggesting the BMP signalling pathway a possible target for lithium action in these cells. We further investigated the mode of lithium action in pluripotent C2C12 cells stably transfected with a BMP-2 expression vector.

Disclosures: ***M. Murshed****, None.*
This study received funding from: McGill University.

SA072

Effects of Odanacatib on Bone Mass and Turnover in Estrogen Deficient Adult Rhesus Monkeys. T. Cusick, B. Pennypacker, D. Kimmel. Molecular Endocrinology/Bone Biology, Merck & Co, West Point, PA, USA.

Odanacatib (ODN) is a selective inhibitor of Cathepsin K (CatK) in non-human primates (NHPs) and humans. Inhibition of CatK may be a promising new mechanism for treating osteoporosis. Rhesus monkeys (aged 13-19yrs) were ovariectomized (OVX), randomized by spinal bone mineral density (LVBMD), and immediately started on ODN (0 [N=11], 6 [N=8], or 30 [N=10] mg/kg, qd, PO) in 0.5% methocel for 21 months. DXA of spine (LV), femoral neck (FN), and total hip (H) (BMD, g/cm^2; BMC, bone mineral content, g) was done quarterly. Pharmacokinetic studies were used to determine ODN exposure. Dual fluorochrome labeling (tetracycline PO; 20mg/kg; 15d interval) was given just before necropsy. Left femurs and the first three lumbar vertebrae (LV1-3) were fixed in 70% ethanol.
Unstained 8m parasagittal sections of LV2 (trabecular), and 100µm cross-sections (cortical) at 4cm from the proximal end of the femur (PF) were prepared and evaluated for double label, single label, and distance between labels, using fluorescent microscopy. Data collection was done on coded specimens. Cortical thickness (Ct.Th) was measured (PF). Mineralizing surface (MSBS, %), mineral apposition rate (MAR, µm/d), and surface-based bone formation rate (BFRBS, mm^2/mm/yr) were calculated.
ODN exposure was 2.2 and 3.8 µM-24hr. Baseline LVBMD differed little among the groups. Ending LVBMD was 11% and 17% higher than OVX+0; HBMD was 10% and 16% higher in the ODN groups. FNBMD was 11% and 12% higher than OVX+0 in the ODN groups. LVMSBS was not affected by 6mg/kg/d ODN, but was ~80% lower with 30mg/kg/d ODN than OVX+0, while LVMAR was unaffected. LVBFRBS was not affected by 6mg/kg ODN, but was ~75% lower with 30mg/kg ODN than in OVX+0. PFMSBS was not affected by 6mg/kg ODN, and tended to be higher with 30mg/kg ODN than in OVX+0; PFMAR was unaffected. PFBFRBS tended to be higher with 30mg/kg ODN. Ct.Th was not affected by 6mg/kg ODN and tended to be higher with 30mg/kg ODN than in OVX+0.
ODN treatment at 6 and 30mg/kg/d in NHPs causes higher bone mass in osteoporotic fracture sites in humans. ODN decreases bone formation activity in vertebral body trabecular bone only at 30mg/kg/d. These data indicate that ODN increases bone mass in adult NHPs at doses where bone formation in trabecular regions appears unaffected by histomorphometric measures. ODN treatment may also be associated with increased periosteal formation rate and cortical thickness at the hip.

Endpoint/Group	OVX+0 (N=11)	OVX+6mg/kg ODN (N=8)	OVX+30mg/kg ODN (N=9)	ANOVA (P=)
LVBMD (g/cm^2)	0.665±0.068	0.731±0.108*	0.771±0.098**	0.040
HBMD (g/cm^2)	0.653±0.066	0.745±0.079**	0.771±0.082***	0.007
LVMS/BS (%)	2.75±2.26	2.21±1.60	0.64±0.65**	0.033
LVBFRBS (mm^2/mm/yr)	9.88±8.77	8.52±7.00	2.20±2.71*	0.057
PFMS/BS (%)	16.0±12.2	16.9±13.3	27.6±16.2+	0.202
PFBFRBS (mm^2/mm/yr)	0.125±0.088	0.095±0.086	0.205±0.150+	0.175

Mean±SD; +(P=0.1); *(P<.05); **(P<.01); ***(P<.001) vs. OVX
H-hip, LV-spine, CF-central femur, FN-femoral neck, PF-proximal femur

Disclosures: ***T. Cusick****, None.*

SA073

Bone Effects of Odanacatib in Adult Ovariectomized Rabbits. B. L. Pennypacker, T. E. Cusick, D. B. Kimmel. Molecular Endocrinology and Bone Biology, Merck and Co., West Point, PA, USA.

Cathepsin K (CatK), a cysteine protease highly expressed in osteoclasts, degrades type I collagen. A CatK inhibitor may be useful for treating osteoporosis. The objective was to evaluate bone mineral density (BMD) and bone turnover in surrogate osteoporotic fracture sites in ovariectomized (OVX) rabbits treated for 28 weeks with CatK inhibitor, Odanacatib (MK-0822).
New Zealand White rabbits (age 7mos) were randomized by spine BMD (DXA, Hologic QDR4500A) and body weight into five groups (N=11-13): Sham-OVX (Sh) + control diet 2031C (Harland Teklad), OVX+ control diet, OVX with 0.0016% dietary MK-0822, OVX with 0.004% dietary MK-0822, or (5) OVX + control diet, treated with alendronate (ALN, 0.3 mg/kg SC 2X/wk). ALN and special diets were started 5-7 days following OVX surgery. Pharmacokinetic studies estimated AUC at ~4 and ~ 9µm·24hr for the 0.0016% and 0.004% diets, respectively. In vivo dual calcein labeling (8 mg/kg, SC) was given on the tenth and third days before necropsy. Lumbar vertebrae (LV) 2-5 and left femurs were excised and fixed in 70% ethanol. The proximal left femur and LV3 were DXA-scanned. LV3 was embedded and sectioned parasagitally at 5µm and analyzed for cancellous bone volume (BV/TV), mineralizing surface, mineral apposition rate (MAR), and bone formation rate (BFR/BS). 100µm cross-sections of the mid-femur were analyzed for cortical thickness, endocortical (e) and periosteal (p) mineralizing surface (MS/BS), and number of double labeled Haversian systems (HS)/mm^2.
Significant OVX-induced BMD decline (-8.7%, p<0.05) occurred at LV3 after OVX. This BMD loss was prevented in both MK-0822 groups and by ALN. BMD was significantly higher in the total hip with MK-0822 and ALN than in OVX+0. BV/TV of LV3 was significantly higher (+13.4%, P<.05) with MK-0822 vs. OVX (P<.04) Mineralizing surface (MS/BS, %) was lower with ALN treatment vs. OVX+0, but was the same or higher than OVX with MK-0822.
These results suggest that estrogen deficiency bone loss was prevented by Cat K inhibition and ALN after seven months. However, CatK inhibition was accompanied by no suppression of bone formation, while ALN was associated with inhibition of bone formation. This may represent either transient or permanent temporal decoupling of formation from resorption in the bone remodeling process, and may differentiate the Cat K mechanism from bisphosphonates.

Variable (N)	Sham (12)	OVX (12)	OVX+4 (13)	OVX+9 (11)	OVX+ALN (10)
LV3 BMD	394.9±11.9	360.3±12.5†	401.3±10.5*	426.6±10.1**	409.1±15.0*
Total Hip BMD	362.7±8.7	357.4±7.6	380±8.9*	385.5±6.2*	393.3±9.0*
LV3 BV/TV	23.2±1.0	21.2±1.0	23.7±2.1	26.3±1.9*	22.4±1.4
LV3 MS/BS	8.0±1.8	10.0±2.3	15.0±2.1†	9.5±1.8	5.5±1.7
LV3 BFR/BS	64.3±16.5	79.3±18.1	120.5±18.5	65.0±13.1	56.8±24.1
Cortical thickness	1.25±.037	1.17±.038	1.188±.035	1.30±.06	1.38±.081*
eMS/BS	11.72±3.06	14.35±3.38	16.82±2.18	16.70±1.9	5.85±.85*
DL Haversian remodeling units	0.583±.23	0.75±.25	1.31±.37	1.3±.37	0.1±.32

*compared to OVX P < 0.05,
** compared to OVX P < 0.001
† compared to Sham P < 0.05

Disclosures: ***B.L. Pennypacker****, None.*

SA074

See Friday Plenary number F074.

SA075

Inhibition of MMP Activity Delays Bone Repair Whereas Inhibition of Osteoclast Function and Formation Does Not. M. M. McDonald*, K. Mikulec*, L. Peacock*, T. Lah*, D. G. Little*. Orthopaedic Research, The Children's Hospital Westmead, Sydney, Australia.

Osteoclasts are associated with all stages of bone repair, including the removal of the soft cartilaginous callus. It is unclear whether specific osteoclast mediated resorption or MMP secretion by osteoclasts and other cells are most important in this process. We designed a fracture healing experiment to dissociate osteoclastic and MMP functions during repair.
In a rat closed femoral fracture model MMI270, an MMP inhibitor was administered subcutaneously (s.c.) twice daily at 120mg/kg while Osteoprotegerin (OPG) at 10mg/kg, Clodronate (CLOD) at 45mg/kg, Zoledronic Acid (ZA) at 0.01mg/kg, or Saline were administered s.c. twice weekly. Dosing continued throughout the entire repair process. Outcomes were assessed at 2, 4 and 6 weeks post fracture. This study design dissociates osteoclast inhibition (the Bisphsphonates ZA and CLOD) from inhibition of osteoclastogenesis (OPG) and global MMP inhibition (MMI270).
MMI270 treatment led to decreased rates of radiographic union compared to Saline ($p<0.01$, Fishers exact test). MMI270 treatment produced a union rate of just 9% at 4 weeks and 30% at 6 weeks. In contrast, all other treatment groups showed equivalent union rates with 70-91% at 4 weeks and 100% union by 6 weeks.
QCT scans revealed a 26% reduction in fracture callus bone mineral content (BMC) and a 42% reduction in callus bone volume (volume) in MMI270 treated samples compared to Saline at 2 weeks ($p<0.01$). At 4 weeks a 26% reduction in callus volume remained ($p<0.05$), and by 6 weeks MMI270 had equivalent BMC and volume to Saline. Both ZA and OPG treatment showed increases in callus BMC (54-97%) and volume (44-66%) at 4 and 6 weeks post fracture ($p<0.01$). CLOD produced smaller but significant increases in both BMC (30%) and volume (35%) over Saline at 4 weeks only ($p<0.01$).
In conclusion, MMP inhibition led to delays in endochondral fracture union whereas OPG, ZA and CLOD did not. MMP inhibition also showed reduced callus BMC and volume, while OPG, ZA and CLOD treatment all produced significant increases in callus BMC and volume ($p<0.01$).
These results suggest that MMP activity rather than osteoclast function may be crucial to achieving bony union in this rat fracture model. Osteoclast function and formation may therefore be redundant during initial endochondral bone repair.

***Disclosures:* *M.M. McDonald**, None.*
This study received funding from: NHMRC.

SA076

AP-1 and Mitf Interact with NFATc1 to Stimulate Cathepsin K Promoter Activity in Osteoclast Precursors. M. Pang*, W. Balkan*, M. Rodriguez*, M. Hernandez*, B. R. Troen. Miami VAMC/GRECC and Geriatrics Institute, University of Miami Miller School of Medicine, Miami, FL, USA.

Cathepsin K (CTSK) is a secreted protease that plays an essential role in osteoclastic bone resorption and osteoporotic bone loss. We have previously shown that AP-1 stimulates CTSK promoter activity and that proximal NFATc1-binding sites play a major role in the stimulation of CTSK gene expression by receptor activator of NFκB ligand (RANKL). We undertook transfection analysis and nuclear factor binding studies to investigate AP-1, NFATc1, and microphthalmia-associated transcription factor (Mitf) cooperativity in the regulation of CTSK transcription. These experiments were carried out in RAW 264.7 cells, which can be readily differentiated to osteoclasts upon RANKL stimulation. Transfection of the individual factors with truncated CTSK promoter-luciferase plasmids significantly stimulated CTSK promoter activity. Cotransfection with combinations of two of the factors (AP-1+NFATc1, AP-1+Mitf, Mitf+NFATc1) dramatically enhanced CTSK promoter activity, with Mitf+NFATc1 producing the largest induction of CTSK promoter activity. Moreover, triple cotransfection of AP-1, NFATc1, and Mitf synergistically stimulated CTSK promoter activity well above levels observed with the double transfections. Cotransfections with dominant negative c-fos markedly inhibited both basal and stimulated CTSK promoter activity, suggesting that AP-1 plays an integral role in transcription of the CTSK gene. Deletion of the 4-bp core elements from three NFATc1 binding sites in the proximal CTSK promoter completely abrogated the response to NFATc1, but did not affect the stimulation by AP-1 or Mitf, either individually or together. Electrophoretic mobility shift assays and analysis of DNA-protein interactions using the Factor Finder system followed by western blots revealed that NFATc1, AP-1, and Mitf bind to similar regions of the CTSK promoter. While AP-1, Mitf, and NFATc1 can independently stimulate CTSK promoter activity, we now demonstrate that these factors cooperate intimately to regulate transcription of the CTSK gene by acting upon the proximal promoter region. Studies are under way to assess the direct DNA-protein interactions of the proximal promoter and thereby more fully elucidate the critical and multiple nuclear factor regulation of transcription of the CTSK gene.

***Disclosures:* *M. Pang**, None.*
This study received funding from: Department of Veterans Affairs and Indial Trail Foundation.

SA077

Identification of Osteoclast Lysosomal Proteins by Mass-Spectrometry. H. Zhao[1], R. D. LeDuc*[2], Y. Ito[1], J. Chappel*[1], R. Townsend*[2], S. L. Teitelbaum[1], F. P. Ross[1]. [1]Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, MO, USA, [2]Proteomic Core Facility, Washington University School of Medicine, St. Louis, MO, USA.

Osteoclasts (OCs) secrete hydrochloric acid to dissolve bone mineral, a process that is mediated by a vacuolar-type proton pump and charge-coupled chloride channel, and release the lysosomal acidic hydrolase cathepsin K to digest the organic matrix of bone. Accumulating evidence indicates that OC secretion to the ruffled border is through regulated lysosomal exocytosis. Consistent with this notion, we have shown that Synaptotagmin VII, a calcium sensor protein that regulates exocytosis, is a key molecule regulating the fusion of lysosomes with the ruffled border membrane and osteoclast function. In order to understand better the molecular mechanisms governing OC lysosome biogenesis, trafficking, and secretion, we set out to identify the protein profile of OC lysosomes by mass-spectrometry (MS). Bone marrow macrophages were transduced with a retroviral vector expressing FLAG tagged lysosome membrane protein, LAMP-2. The cells were cultured with M-CSF and RANKL for 5 days to generate mature OCs. A lysosome-enriched fraction was collected from an iodixanol density gradient ultracentrifugation. To achieve higher purity, lysosomes were immuno-purified with magnetic beads coated with anti-FLAG monoclonal antibody. By this method, intact vesicles (lysosomes) on the beads were isolated, as demonstrated by transmittion electron microscopy. Western blots of bead elution showed that LAMP-2 and Cathepsin K were recovered, whereas the mitochondria proteins (cytochrome c), cytoskeletal proteins (actin and tubulin) and early endodomal proteins (EEA1), were removed. The proteins bound to the magnetic beads were then separated by SDS-PAGE and a total of 96 gel punches were trypsin digested and analyzed by MS. The raw data were searched with Mascot. More than 300 proteins have been identified, which belong to the families of small GTPases, vesicular/protein trafficking regulators, molecular channels, lysosomal membrane proteins and acidic hydrolases, molecular motors and cytoskeletal regulators. Importantly, several lysosomal proteins, functionally important for OCs, have also been identified, including cathepsin K, LAMP-1/2, a3 and v_0 d2 subunits of vacuolar ATPases, and rab 7. Thus, we have established an experimental system for proteomic analysis of protein profiles in OC lysosomes, providing the capacity to identify novel proteins regulating bone resorption. Further functional characterization of these novel proteins will provide new insights into the molecular mechanisms of OC secretion.

***Disclosures:* *H. Zhao**, None.*
This study received funding from: NIH.

SA078

Expression of NF-κB Ligand and Regulation of Osteoclastogenesis by Bone Marrow Adipocytes. A. Hozumi*. Orthopaedics, Ngasaki University, Nagasaki, Japan.

Objective.: NF-κB Ligand (RANKL), Osteoprotegerin (OPG) and macropharge colony stimulating factor (MCSF) are potent regulator of osteoclastogenesis. Adipocytes and osteoblasts share a common origin, and several studies reported adipokines have close relationship in the bone metabolism. Now we examined whether RANKL, OPG and MCSF were secreted from adipocytes. This study examined the ability of bone marrow adipocytes to support osteoclast formation and function in vitro.
Materials and Methods: The bone marrow fluids were obtained from 10 individuals at prosthetic insertion. The mRNA expression of RANKL, OPG and MCSF was measured by real time RT-PCR and examine the effect of glucocolticoid. Furthermore we examined osteoclastgenesis of osteoclastprecursor cell in co-culture system with bone marrow adipocytes. Osteoclast differentiation was assessed by expression of titrate-resistant acid phosphatase (TRAP).
Results: RANKL, OPG and MCSF messenger ribonucleic acid (mRNA) expression are confirmed in all indvisual. RANKL/OPG ratio was significantly increased treated by dexamasasone compared to the conrol group in bone marrow adipocytes. MCSF expression was not significantly altered in both cell group. RANKL/OPG ratio was significant at 10^{-7} mol/L dexamethasone in mRNA levels and it was time-dependent manner up to 24 hr. Osteoclast differentiation and maturation ware undergone when cocultured with bone marrow adipocytes and significantly stimulated by dexamethasone (10-7mol/l).**Conclusion:** Our studies demonstrated for the first time that primary bone marrow mature adipocytes can support osteoclasts-like cell formation and function in vitro.

***Disclosures:* *A. Hozumi**, None.*

SA079

See Friday Plenary number F079.

SA080

Expression and Function of Synoviolin in Human Osteoclastogenesis. B. Merle*[1], P. D. Delmas[1], P. Miossec*[2], M. L. Toh*[2]. [1]INSERM U831, Université de Lyon, LYON, France, [2]Unité Mixte Hospices Civils de Lyon-BioMérieux, LYON, France.

Synoviolin, is an anti-apoptotic E3 ubiquitin ligase implicated in Rheumatoid arthritis (RA) synovial hyperplasia, however its role in bone destruction is less well understood. Transgenic mice overexpressing synoviolin show ubiquitous expression of synoviolin in various tissues including bone. The aim of our study was to investigate if synoviolin is expressed by human osteoclasts and implicated in their function *in vitro.*
Human osteoclasts were differentiated from human peripheral blood monocytes in the presence of RANK-L and M-CSF. Osteoclast differentiation was assessed by TRAP staining and TRAP activity in conditioned media. Synoviolin expression was measured by real-time PCR, Western Blotting and immunostaining. Synoviolin inhibition was achieved by RNA interference during osteoclastogenesis. Osteoclast function was determined by analysis of pit formation and assessment of type I collagen degradation products by ELISA.
Synoviolin was expressed in human monocytes and increased during osteoclastogenesis. Synoviolin mRNA and protein expression was 2 to 3 fold higher in mature osteoclasts compared to monocyte precursors. Immunohistochemistry staining demonstrated that synoviolin was localised to the perinuclear region in pre-osteoclasts and fully differentiated osteoclasts. Synoviolin inhibition during osteoclastogenesis dramatically reduced osteoclast formation as shown by TRAP staining and activity in the cell supernatants. In parallel, resorptive activity was reduced as measured by pit formation and the release of collagen fragments in cultured medium. Finally, synoviolin expression was inhibited by IL-4 and oestrogen during osteoclast differentiation.
In conclusion, we show that synoviolin is expressed by human osteoclasts and involved in the differentiation, survival and activity of osteoclasts *in vitro*. Overall, our findings suggest that synoviolin may be involved in the inflammatory osteoclast-mediated bone resorption observed in RA, and in the alterations of bone remodeling associated with metabolic bone diseases. In addition, oestrogen may have a protective effect on bone resorption at least in part through decreasing osteoclast survival.

***Disclosures: B. Merle**, None.*

SA081

See Friday Plenary number F081.

SA082

A Novel Role of L-Serine for the Activation of Receptor Activator of Nuclear Factor Kappa B Ligand (RANKL)-RANK Signaling Machinery in Osteoclastogenesis *in vitro*. T. Ogawa, T. Sakai*, A. Bahtiar*, N. Ishida-Kitagawa*, T. Takeya*. Graduate School of Biological Sciences, Nara Institute of Science and Technology, Ikoma, Nara, Japan.

The induction of two key transcription factors, c-Fos and nuclear factor of activated T cells c1 (NFATc1/NFAT2), is known to be essential for osteoclastogenesis. We previously found, using mouse macrophage cell line RAW264 cells, that culture at high cell density blocked progression to the multinucleated cell stage induced by stimulation with receptor activator of nuclear factor kappa B ligand (RANKL). This finding eventually led us to the identification of NFATc1 as a key regulator of osteoclastogenesis. We subsequently confirmed the cell density-dependent suppression of osteoclastogenesis in a bone marrow cell system, and extended the analysis further. In comparative analysis of conditioned medium from high and low cell density cultures, we noticed the indispensability of L-serine (L-Ser), one of seven non-essential amino acids in culture medium, as a pivotal factor for the expression of NFATc1 induced by RANKL. Namely, culture at high cell density caused a depletion of L-Ser in the medium. The level of NFATc1 protein was found to be correlated with the presence of L-Ser in a concentration dependent manner. In contrast, D-Ser, an enantiomer of L-Ser, showed no NFATc1-inducing activity. We further examined the effect of L-Ser depletion on the upstream events of NFATc1 induction after RANKL stimulation. It was observed that, in the absence of L-Ser, activation of MAPK pathways and c-Fos induction triggered by RANKL stimulation were also hampered. L-Ser was subsequently found to be necessary for the expression of RANK, the receptor for RANKL. In the absence of L-Ser, the expression of RANK protein was decreased to undetectable level within 8 hours. The level of *rank* mRNA was suppressed in bone marrow cells under L-Ser depleted condition, whereas downregulation of *rank* mRNA was not evident in RAW264 cells. Furthermore, forced expression of RANK as well as NFATc1 using retrovirus vectors could compensate for the depletion of L-Ser and resume the progression to the multinucleated cell stage. These results demonstrate a novel but crucial role for L-Ser that as a prerequisite factor for RANKL-induced osteoclastogenesis in *vitro*.

***Disclosures: T. Ogawa**, None.*

SA083

See Friday Plenary number F083.

SA084

Pyrophosphates Stimulates Osteoclast Differentiation and Bone Resorption. S. Abdelmagid*, A. Zajac*, I. Salhab*, H. Nah. Plastic and Reconstructive Surgery, Children Hospital of Philadelphia, Philadelphia, PA, USA.

Craniometaphyseal dysplasia (CMD) is a rare disease characterized by overgrowth of facial and cranial bones and abnormal flaring of metaphyses of long bones. Although the skeletal phenotype suggests that the bone remodeling process may be disregulated in CMD, the cellular mechanisms underlying the CMD phenotype is not known. The autosomal dominant form of CMD has recently been linked to loss of function mutations in ANKH, a transmembrane protein involved in the transport of pyrophosphate (PPi). Therefore, in this study we determined the effect of PPi on bone remodeling, using *ex vivo* murine neonatal calvarial organ and bone marrow cell cultures. Our data show that exogenous PPi (0.1-1mM) stimulates a calcium release in the media of calvarial organ cultures maintained for 3 days ($p<0.05$) and TRAP staining of calvarial bones ($p<0.01$) in a dose-dependant manner, indicating that PPi stimulates bone resorption by osteoclasts. The PPi effect on bone resorption was not inhibited by either alkaline phosphatase inhibitor (Levamisole) or Pi transport inhibitor (PFA), indicating that the effect of PPi on bone resorption is not dependent on inorganic phosphates hydrolyzed from PPi. On the other hand, PPi stimulation of calcium release was abolished by an NFκB inhibitor, indicating that the PPi effect on bone resorption is NFκB-dependent. Cell culture data show that PPi also stimulates proliferation of bone marrow-derived osteoclast precursors ($p<0.01$) and RAW 264.7 pre-osteoclast cell line ($p<0.01$) in a dose-dependant manner. Nevertheless, PPi alone was not able to induce differentiation of bone marrow-derived pre-osteoclasts. Instead, pretreatment for 48 hours with PPi (0.1-1mM) accelerated differentiation of the osteoclast precursors under a differentiation permissive condition, as measured by cell TRAP activity ($p<0.01$), number of TRAP positive osteoclasts ($p<0.05$), and nuclei fusion index ($p< 0.05$), suggesting that PPi enhances the differentiation potential of pre-osteoclasts. Taken together, our data suggest that PPi stimulation of bone resorption in calvarial organ cultures may be mediated in part by its priming effect on pre-osteoclasts for cell differentiation.

***Disclosures: S. Abdelmagid**, None.*

SA085

See Friday Plenary number F085.

SA086

Inhibin Directly Targets Suppression of Isolated Human Osteoclast Precursor Development and Activity. K. M. Nicks, N. S. Akel*, L. J. Suva, D. Gaddy. Physiology and Biophysics & Orthopaedic Surgery, University of Arkansas for Medical Sciences, Little Rock, AR, USA.

Serum Inhibin A (InhA) is a better predictor of bone formation and resorption markers than either follicle stimulating hormone (FSH) or bioavailable E2 in premenopausal women. Regardless of changes in sex steroids or FSH, increased bone turnover markers correlate with decreased serum InhA and InhB levels in pre-, peri-, and post-menopausal women, consistent with our *in vitro* demonstration that Inh suppresses osteoblast development of hMSCs and OCL development of human peripheral blood mononuclear cells (hPBMCs). To determine if InhA suppression of osteoclastogenesis is mediated by direct effects on OCL precursors, OCL cultures of either unselected hPBMCs, or a sub-population of OCL precursor cells (purified CD14+ hPBMCs) and treated with RANKL, mCSF +/- InhA and/or FSH were initiated. In cultured CD14+ OCL precursors, FSH significantly increased RANKL-dependent OCL differentiation whereas InhA significantly suppressed OCL differentiation. However, in heterogeneous PBMCs, RANKL-dependent OCL differentiation was significantly inhibited by either InhA or FSH. The effect of InhA was significantly more potent than FSH. In both CD14+ and PBMC cultures, RANKL-induced OCL formation in response to InhA + FSH was not significantly different from InhA alone, demonstrating the dominant effect of InhA to block any FSH effect. Since InhA was found to directly target CD14+ OCL precursor development, cells were cultured on dentine slices to measure OCL activity. InhA treatment significantly decreased bone resorption and prevented OCL migration, even in the presence of FSH. The signaling receptors mediating InhA and FSH action were determined using immunofluorescence. CD14+ OCL cultures were initiated and harvested on days 1 and 7. RANK expression was observed on both days 1 and 7, unlike the FSH receptor and the Inhibin-specific receptor, betaglycan, which were not observed on day 1 of culture but were present by day 7. Also present at day 7 were Type II receptors for BMP and Activin (BMPRII, and ActRIIA, but not ActRIIB). These data suggest that isolated OCL precursor cells become capable of responding to both InhA and FSH only after RANKL initiation of OCL development. Although FSH can enhance OCL development when CD14+ precursors are cultured, InhA dominantly suppresses. In fact, InhA suppresses OCL development, migration and bone resorption in the presence or absence of FSH. Furthermore, in heterogeneous PBMC cultures, which represent a more physiological environment in which to test the integrated role of these hormones, the effect of InhA and FSH (alone or in combination) is to suppress OCL development and activity.

***Disclosures: K.M. Nicks**, None.*
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SA087

See Friday Plenary number F087.

SA088

RANKL-mediated Osteoclast Lineage Commitment Dictates the Role of Lipopolysaccharides in Osteoclast Differentiation. J. Liu*[1], S. M. Michalek*[2], X. Feng[1]. [1]Pathology, Univ. of Alabama at Birmingham, Birmingham, AL, USA, [2]Microbiology, Univ. of Alabama at Birmingham, Birmingham, AL, USA.

Lipopolysaccharides (LPS), common bacteria-derived products, are recognized as a key factor implicated in periodontal bone loss. However, the role of LPS in osteoclast (OC) formation still remains controversial. The prevailing view is that LPS stimulate OC formation, which is not only supported by various contemporaneous studies, and yet consistent with the role that LPS are presumed to play in periodontal bone loss. Two groups, however, have shown that LPS play an inhibitory role in OC formation. Here, we carried out independent and thorough studies to address the controversy. We treated primary bone marrow macrophages (BMMs) from 6-week-old mice with M-CSF (44ng/ml) and different doses of LPS (5ng/ml-10ug/ml). Our data indicate that LPS and M-CSF are unable to stimulate OC formation. Next, we determined whether LPS can promote OC formation in the presence of M-CSF (44ng/ml) and RANKL (100ng/ml). In doing so, we added LPS at the beginning of the assays. We found that LPS inhibit OC formation in a dose-dependent manner (>95% at 5ng/ml) in the presence of M-CSF (44ng/ml) and RANKL (100ng/ml). However, when we treat BMMs with M-CSF (44ng/ml) and RANKL (100ng/ml) for as short as 12 hrs, LPS promoted OC formation from these committed BMMs in the presence of M-CSF and RANKL. Moreover, we found that LPS and M-CSF, without RANKL, are sufficient to promote OC formation from the RANKL-primed BMMs. Together, these findings support that RANKL-mediated OC lineage commitment dictates the role of LPS in OC formation. To further address the issue, we treated BMMs with M-CSF (44ng/ml) and RANKL (100ng/ml) for 24hrs. We then removed RANKL and cultured the cells with M-CSF alone (44ng/ml) for 12hrs, 24hrs, 2d and 3d. At each time point, BMMs were lifted and re-plated to perform OC formation assays with M-CSF, RANKL and LPS. Even 3d after the RANKL pretreatment, BMMs are still able to form OC in response to M-CSF, RANKL and LPS, revealing that the OC lineage commitment by RANKL is long-term, not transient. Finally, given that osteoblasts are indirectly involved in LPS-mediated OC formation by producing RANKL in response to LPS, we investigated the role of LPS in OC formation in co-culture system containing osteoblasts and BMMs pretreated by RANKL (100ng/ml) for 24hrs or untreated BMMs as control. The co-cultures were treated with or without LPS (10ng/ml). While the co-cultures containing committed BMMs formed OC in response to LPS, control co-cultures failed to do so. These results further support that RANKL-mediated OC lineage commitment determines the action of LPS in OC formation.

Disclosures: ***J. Liu****, None.*

SA089

See Friday Plenary number F089.

SA090

S100 Protein Directly and Indirectly Affects RANKL-stimulated Osteoclastogenesis. T. Yoshida, A. Flegler*, P. H. Stern. Department of Molecular Pharmacology and Biological Chemistry, Northwestern University Feinberg School of Medicine, Chicago, IL, USA.

Diabetic osteopenia is one of the complications associated with Diabetes Mellitus, and Advanced Glycation Endproducts (AGEs) are thought to be involved in this disease. It was also reported recently that the expression of S100A4 protein, one of the ligands for receptor of AGEs (RAGE), is regulated in the course of osteoblast differentiation and this regulation is crucial for normal differentiation. However, it is still unclear how S100 protein affects bone metabolism. We were therefore interested in determining how S100 protein affects RANKL- stimulated osteoclastogenesis, either directly or indirectly through cytokines secreted from osteoblasts.
We first determined whether there was a direct effect of S100 on osteoblast proliferation and osteoclastogenesis. Studies were carried out with MC3T3-E1 pre-osteoblastic cells and RAW 264.7 mouse monocyte/macrophage lineage cells, which differentiate into multinucleated mature osteoclasts in the presence of receptor activator of nuclear factor-κB ligand (RANKL). MC3T3-E1 cells were incubated for 3, 7 or 14 days and proliferation rate was determined by MTT assay. S100 significantly inhibited osteoblast proliferation at day 14 but not at the shorter time points. RAW 264.7 cells were incubated for 7 days with human soluble RANKL (sRANKL) and tartrate resistant acid phosphatase (TRAP) activity and TRAP-positive multinucleated cells were measured as markers of osteoclastic differentiation. Osteoclastic differentiation was significantly inhibited by S100. Proliferation, as assessed by MTT assay, was also decreased by S100. There was a trend towards a decrease in the number of TRAP-positive multinucleated cells, but this was not statistically significant. We then determined whether S100 protein could affect osteoclastogenesis indirectly, i.e., through altering osteoblastic secretion of a stimulator of osteoclast differentiation such as RANKL, M-CSF, or the ligand of osteoclast- associated receptor (OSCAR). MC3T3-E1 cells were incubated with or without S100 for up to 15 days. Culture medium was then changed to medium without S100 and incubated with the MC3T3-E1 cells for an additional 6h. This medium was used for treatment of the RAW cells. Unexpectedly, media from S100 treated MC3T3-E1 cells significantly promoted the proliferation of RAW cells, but no indirect effect on differentiation was seen. Thus, osteoclastogenesis could be regulated directly and indirectly by S100, possibly secreted from osteoblasts by an autocrine process to regulate osteoclastogenesis, and this regulation might be involved in the progression of diabetic osteopenia.

Disclosures: ***T. Yoshida****, None.*
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SA091

See Friday Plenary number F091.

SA092

Neutrophil-Like PLB-985 Cells Induce Differentiation of Human Blood Monocytic Precursors into Functional Osteoclasts via a Receptor Activator of NFKappa-B ligand (RANKL)-Dependent Mechanism. A. Chakravarti*, P. E. Poubelle. Medicine, Université Laval, Québec, QC, Canada.

Focal bone loss in the inflammatory arthritides begins early in the disease process and can contribute to patient morbidity. This excessive bone resorption is due to increased activity of osteoclasts that require receptor activator of NF-Kappa B-ligand (RANKL) to function. Neutrophils are the predominant infiltrating cells at this stage. Though neutrophil presence is linked to disease severity, their role in the evolution of bony lesions is not clear. In the present study we used human myeloid cell line PLB-985, which can be induced to express a neutrophil-like phenotype, as a model for study of neutrophil-osteoclast interactions. We demonstrated that these neutrophil-like cells directly activate osteoclasts through RANKL expressed on the surface of PLB-985 cells. *In vitro* co-culture of human pre-osteoclasts, derived from circulating monocytes, with fixed PLB-985 cells induced differentiation of mononuclear osteoclast precursors into large multinucleated cells. These multinucleated cells were positive for the osteoclast specific enzymatic marker, tartrate-resistant acid phosphatase. Importantly, when co-cultures were conducted on devitalized dentine slices, well-defined bone resorption pits were observed after toluidine blue treatment. Thus, the neutrophil-like cells stimulated resorptive activity in mature osteoclasts. Gene knockdown of RANKL following nucleofection of RANKL specific siRNA in PLB-985 cells effectively diminished RANKL RNA, as seen by QRT-PCR, and surface protein expression evaluated by flowcytometric analysis. Induction of osteoclast differentiation and activity was also significantly diminished in co-culture assays of osteoclasts with transfected PLB cells, proving that PLB-derived RANKL was specifically responsible for the stimulation of osteoclasts. In conclusion, we propose that neutrophil-derived RANKL might have an important role, not only in the inflammatory component of the arthritides, but also in the early skeletal involvement that characterizes these conditions. Our work is the first to demonstrate functional interactions between the two cell types: neutrophil and the osteoclast.

Disclosures: ***A. Chakravarti****, None.*
This study received funding from: CIHR/IRSC.

SA093

See Friday Plenary number F093.

SA094

Effect of Mesenchymal Stem Cells in Coculture with Osteoclasts: A Contact Mediated and Dose-Dependent Effect? C. Janeiro, D. Vashishth. Biomedical Engineering, Rensselaer Polytechnic Institute, Troy, NY, USA.

Osteoblasts, which are derived from stem cells, supply factors that positively influence the differentiation of osteoclast precursors to osteoclasts (OCLs) through signaling pathways [1]. However, the effect of mesenchymal stem cells (MSCs), which reside in bone marrow in close proximity to osteoclast precursors, has not been examined. To determine the mechanism, a set of experiments was devised to determine whether the effect is contact or signaling mediated.

Human adult osteoclast precursor cells and human mesenchymal stem cells (both from Cambrex Poietics™) were cocultured at varying densities (OCLs remained constant at 6000 cells/well, while MCSs were varied from 0-4200 cells/well) following supplier's instructions under standard cell culture conditions for up to 14 days, in standard 96 well plates (contact-mediated) and Transwell™ plates (signaling-mediated) alone and in the presence of bone slices. For each coculture variation, the number of differentiated OCLs and the resorption on the bone were analyzed. For each group, contact-mediated and signaling-mediated, the percent change in differentiated OCLs was calculated as a function of the positive control (OCL precursors only). Analysis of select cytokines was also examined.

The percent change of differentiated OCLs decreased linearly with an increase in MSCs only when the cells were allowed contact (Fig 1). For the signaling mediated group, the percentage of differentiated OCLs remained steady throughout the variation of MSC number. The only cytokine that has yielded significant results was TNF-α, displaying a decrease in TNF-α with the addition of OCLs.

These results demonstrate a clear difference between contact mediated and signaling mediated cocultures of OCLs and MSCs. The increase in the number of OCLs with a decrease in MSC number suggests that the MSCs regulate the differentiation of OCLs from hematopoietic stem cells. These findings suggest the age-related decrease in MSC as a plausible mechanicsm of increased OCL differentiation with increased age.

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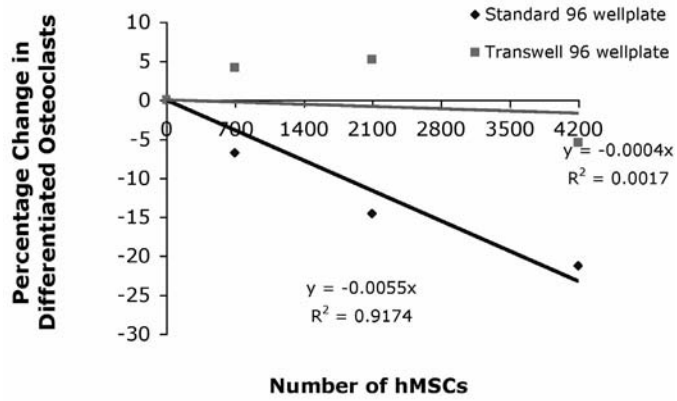

***Disclosures:* C. Janeiro**, *None.*
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SA095

Gastric Proton Pump Inhibitors Failed to Affect Bone Resorption and Formation. T. Nishisho*[1], L. Wang*[1], K. Hata[1], M. Nakanishi*[1], N. Yasui*[2], T. Yoneda[1]. [1]Dept Biochem, Osaka Univ Grad Sch Dent, Suita, Osaka, Japan, [2]Dept Orthop, Univ Tokushima Grad Sch Med, Tokushima, Japan.

Osteoclasts dissolve bone minerals by releasing protons through the vacuolar type proton pump (V-ATPase), making neighboring microenvironments acidic. Inhibitors of the V-ATPase therefore could be pharmacological agents for bone diseases associated with increased osteoclastic bone resorption. On the other hand, a gastric H^+, K^+-ATPase, a family member of the P-ATPase, is highly expressed in parietal cells in stomach and plays central roles in gastric acid secretion. Inhibitors of H^+, K^+-ATPase are the most wildly used drug for the treatment of gastric ulcer, duodenal ulcer and gastroesophageal reflux disease. Of note, a recent clinical study has reported that administration of gastric H^+, K^+-ATPase inhibitors are associated with reduced bone mass and increased fracture rate, raising the possibility that these inhibitors promote osteopenia. However, these studies remain controversial and the effects of H^+, K^+-ATPase inhibitors on bone have not been fully elucidated. In the present study, we investigated the effects of the gastric H^+, K^+-ATPase inhibitors (PPIs) on bone resorption and formation *in vitro* and *in vivo*. To study this, we tested widely-used PPI, rabeprazole and omeprazole. As control we also examined the effects of FR167356, a specific inhibitor for the a3 V-ATPase. Mouse spleen cells were cultured in the presence of M-CSF (30ng/ml) and sRANKL (100ng/ml) for 6 days together with these inhibitors. Tartrate-resistant acid phosphatase-positive multinucleated osteoclast-like cell (TRAP (+) OC) formation and pit formation on dentin slices were inhibited by FR167356 (10^{-8}M-10^{-6}M) in a dose-dependent manner. In contrast, rabeprazole and omeprazole (10^{-8}M-10^{-6}M) showed no effects on TRAP (+) OC formation and bone resorption. Histological examination revealed that rabeprazole and omeprazole failed to inhibit osteoclastic bone resorption in the calvariae in the mouse model of lipopolysaccharide-induced bone destruction. We next examined whether these inhibitors affect bone formation. Both rabeprazole and omeprazole had no effects on BMP2-induced osteoblast differentiation in C3H10T1/2 and C2C12 cells determined by alkaline phosphatase activity. Moreover, no significant effects were observed in the mineralization of primary neonatal mouse calvarial osteoblasts. In conclusion, our results show that PPIs have little direct influences on bone and suggest that yet-unknown mechanism is involved in the pathogenesis of osteopenia in PPI-treated individuals.

***Disclosures:* T. Nishisho**, *None.*

SA096

Urocortin Strongly Suppresses the Formation and Function of Osteoclasts via a Novel Mechanism. C. E. Combs*, K. Fuller, T. J. Chambers, K. M. Lawrence*. Department of Histopathology, St George's Hospital Medical School, London, United Kingdom.

Urocortin (Ucn) is a 40 amino acid peptide, related to the hypothalmic corticotrophin releasing factor (CRF) family which now also includes Ucn 2 and 3. Since its discovery Ucn has been found to be widely distributed in the CNS, digestive, cardiovascular, reproductive, immune and endocrine systems. It has extremely diverse functions including reducing blood pressure in the cardiovascular system and decreasing appetite in the CNS. Recently, it has been implicated as a modulator of the immune/inflammatory system. Of particular interest, Ucn has been shown to be increased in the synovial fluid of patients with rheumatoid arthritis, and reduces inflammation and bone erosion in a mouse model of the disease. Beyond this, nothing is known of the role of Ucn in the pathobiology of bone.

We therefore tested the effect of Ucn on osteoclast formation and function. Ucn showed a dose-dependent inhibition of the number of osteoclast-like cells (Ocl) formed when murine non-adherent bone marrow cells were incubated with RANKL and MCSF. We then tested the effect of Ucn on osteoclast function. To do this, *in vitro* derived Ocl were sedimented onto bone slices, and incubated with/without Ucn. We found strong suppression of bone resorption. To confirm that this was a distinct effect on osteoclastic function rather than formation, we tested the effect of Ucn on actin ring formation. Ucn similarly potently suppressed the formation of actin rings in osteoclasts, in a dose-dependent manner, with 50% inhibition of actin ring formation occurring at $5x10^{-9}$M, and almost complete inhibition at 10^{-7}M.

It is known that activation of CRFR1 or R2 by Ucn results in elevation of either cAMP via Gs, or of IP3/diacylglycerol via Gq. We assessed expression of Ucn and its receptors in bone cells using semi-quantitative RT-PCR. We found that although osteoclasts, macrophages and osteoblasts do not express any of the known CRF receptor subtypes, osteoclasts and macrophages both express Ucn itself. The absence of known receptor types was supported by experiments in which we found that a receptor-antagonist did not block the effects of Ucn on Ocl function or formation. Furthermore, Ucn failed to generate production of either cAMP or cGMP in these cells, and selective PKA/PKC inhibitors did not suppress the anti-resorptive effect of Ucn. We did however note, using confocal microscopy, a rapid intracellular calcium transient in osteoclasts. We conclude that Ucn potently inhibits osteoclasts via a mechanism independent of known CRF receptors. Expression of Ucn by bone cells raises the possibility that it may play an autocrine/paracrine role in the pathophysiology of bone.

***Disclosures:* C.E. Combs**, *None.*

SA097

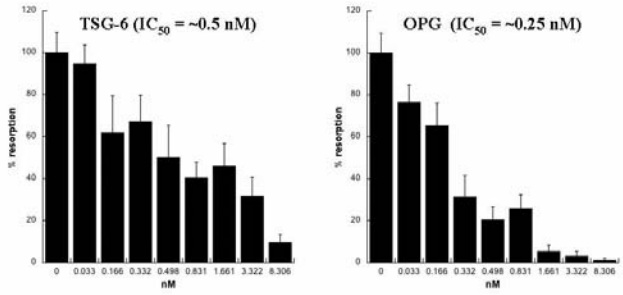
TSG-6 Acts Synergisticly with OPG to Inhibit Bone Resorption. D. Mahoney*[1], C. M. Milner*[2], A. J. Day*[2], A. Sabokbar[1]. [1]Botnar Research Centre, University of Oxford, Oxford, United Kingdom, [2]Faculty of Life Sciences, University of Manchester, Manchester, United Kingdom.

TSG-6 (TNF-stimulated gene-6) is a 35-kDa protein, composed almost entirely of Link and CUB_C domains, that is up-regulated by inflammatory mediators (e.g. TNF, IL-1). To date a wide variety of protein and glycosaminoglycan ligands (e.g. hyaluronan, heparin, bikunin, pentraxin-3 and thrombospondin-1) have been identified as associating with the Link module, whereas only fibronectin has been found to bind to the CUB_C region. Animal models of arthritic disease have indicated that TSG-6 has anti-inflammatory and chondroprotective properties. We have recently demonstrated that recombinant TSG-6 inhibits RANKL-mediated bone resorption by osteoclasts (Mahoney et al., manuscript submitted). The full-length protein had greater inhibitory activity compared to the isolated Link module and the CUB_C domain was essentially inactive. Furthermore, binding analyses revealed that TSG-6 interacts directly with sRANKL, probably at a composite binding surface involving both the Link and CUB_C domains. To further understand the role of TSG-6 as an inhibitor of bone resorption, we sought to (i) compare the potency of TSG-6 to that of OPG and (ii) determine whether TSG-6 can act synergistically with OPG in inhibiting RANKL-mediated osteoclastic bone resorption.

In vitro assays revealed that TSG-6 inhibited RANKL-induced resorption in a dose-dependent fashion, in a similar manner to that observed for OPG; the IC50 of full-length TSG-6 was 0.5nM as compared to 0.25nM for OPG. Furthermore, we have demonstrated that a combination of TSG-6 and OPG significantly inhibited the RANKL-mediated resorption compared to treatment with each protein alone.

These findings indicate that TSG-6 can act synergistically with OPG and as such TSG-6 could have therapeutic potential for the treatment of osteolytic bone disorders.

Figure 1



***Disclosures:* *A. Sabokbar**, None.*
This study received funding from: Arthritis Rheumatism Campaign.

SA098

See Friday Plenary number F098.

SA099

Proteasome Inhibitors Attenuate Osteoclastogenesis and Bone Resorption via the Modulation of RANK-mediated TRAF6, p62 and IκB-α Signaling Cascades. E. S. Ang*[1], N. Pavlos[1], T. Chai*[1], K. Yip*[1], S. Rea*[2], T. Ratajczak*[3], M. Zheng[1], J. Xu[1]. [1]Centre for Orthopaedic Research, The University of Western Australia, Nedlands, WA, Australia, [2]Western Australia Institute for Medical Reseach, The University of Western Australia, Nedlands, WA, Australia, [3]Western Australia Institute for Medical Research, The University of Western Australia, Nedlands, WA, Australia.

Enhanced osteoclast formation and activation becomes manifest in many pathological osteolytic conditions including osteoporosis, Paget's disease and tumor metastasis to bone. Proteasome-mediated pathways regulate diverse signaling cascades that play fundamental roles in cell differentiation and apoptosis. However, the molecular mechanism(s) by which the proteasome pathway regulates osteoclast formation, bone resorption and its potential impact on RANKL-mediated signaling remains to be elucidated.
Using both primary bone marrow monocytes (BMMs) and RAW264.7 cell osteoclastogenic culture systems, we examined the effects of several proteasome inhibitors on osteoclast formation. In addition, we employed bone resorption assays and confocal microscopy to determine the effect(s) of proteasome inhibitors on the function and cytoskeletal architecture of osteoclasts. Immunoblot and immunoprecitation analyses together with luciferase reporter gene assays and immunocytochemistry were used to dissect the molecular mechanism(s) underlying the observed effects of proteasome inhibitors on NF-κB, IκBα, p62 and TRAF6.
We demonstrate that proteasome inhibitors MG-132, MG-115 and Epoxomicin dose-dependently attenuate RANKL-induced osteoclastogenesis, with relative potency Epoxomicin > MG-132 > MG-115 based on the equi-molar concentrations. At high concentrations, proteasome inhibitors were found to induce cellular apoptosis and disrupt F-actin and microtubule organization in osteoclasts. Epoxomicin potently inhibited bone resorption by both BMM-derived osteoclasts and human osteoclast-like cells derived from giant cell tumors of bone. The same proteasome inhibitors were also found to effectively block RANKL-induced NF-κB activation, as well as prolong the proteasome-mediated degradation and targeting of IκBα, p62 and TRAF6.
Collectively, our findings demonstrate that proteasome inhibitors perturb osteoclast formation and function by influencing key RANK-mediated signaling cascades (IκB-α, p62 and TRAF6). We propose that selective proteasome inhibitors might offer potential therapeutic value for the treatment of osteoclast-mediated bone diseases.

***Disclosures:* *E.S. Ang**, None.*
This study received funding from: National Health and Medical Research Council.

SA100

Resorption Mechanism of Hydroxyapatite and ß-Tricalcium Phosphate Coating Layer. D. Lee*, K. Lee*, J. Kim*, C. Lee*, J. Chang*. Orthopaedic Surgery, Asan Medical Center, Seoul, Republic of Korea.

Beta-tricalcium phosphate (β-TCP) coating layer is known to be resorbed much faster than hydroxyapatite(HA), however, there has been no report to explain the exact reason of that to our knowledge. Therefore, we investigated whether the resorption mechanisms of these two compounds are same, if not, what is the difference. Eighty titianium discs with 12mm in diameter and 2mm in thickness were coated with HA(n=40) or β-TCP(n=40) by dip and spin coating method. In each group, the specimens into 2 subgroups respectively; Dissolution (D, n=20) and Osteoclast culture (C, n=20). The coated discs in D group were immersed in the cell culture media for 5 days, whereas, in C group, osteoclast-like cells ($5x10^3$ cells/500μl), which were isolated form human giant cell tumor, were seeded on the specimens and cultured for 5 days. Cultured cells were defined as osteoclast by the determination of osteoclast marker (tartarate-resistant acid phosphatase,TRAP). After immersion or osteoclast culture, the dissolution characteristics of coating layer surface were observed using light microscope (LM) and scanning electron microscope (SEM). In HA-C and β-TCP-C groups, area fraction of resorption lacunae formed by osteoclast was analysed by image analysis to evaluate the activity of osteoclastic degradation. After dissolution test, β-TCP coating layer showed much more cracks and denudation as compared to HA coating layer. In C group, the osteoclasts covering the coating layer were identified on LM and SEM images. Mean area fraction of resorption lacunae in HA-C group was 11.62%, which was significantly higher than that of 0.73% in β-TCP-C group (p=0.001). The resorption mechanisms of HA and β-TCP coating layers were different each other *in vitro* study. The coated β-TCP was degraded mainly by dissolution and separation from implant, on the other hand, the HA coating layer was resorbed by osteoclastic activity.

***Disclosures:* *D. Lee**, None.*

SA101

Human OsteoProgenitor Cell Adhesion and Spreading on Functionalized Titanium Surfaces Followed by Quartz Crystal Resonators (QCM) and Confocal Laser Scanning Microscopy (CLSM). D. Le Guillou-Buffello*[1], R. Bareille*[2], M. Gindre*[3], A. Sewing*[4], P. Laugier*[3], J. Amedee[2]. [1]CNRS UMR7623, Paris, France, [2]INSERM Unité 577, Bordeaux, France, [3]CNRS UMR 7623, Paris, France, [4]Biomet Deutschland GmbH, Berlin, Germany.

The surface characteristics of endosseous implant such as titanium-based biomaterials control considerably the cellular response and subsequently the quality and the quantity of new-formed bone around the implant. A variety of invasive methods exist for quantifying cell adhesion to a potential biomaterial surface (direct counting of labelled cells after fixing or enzymatic detachment, focal contact imaging by confocal microscopy or CLSM). The quartz crystal microbalance (QCM) technique is an attractive in vitro method for real-time characterization of initial cell adhesion with no need for destructive interventions.
The aim of this paper is to monitor cell adhesion under well defined conditions of medium without serum, on different titanium coatings (hydroxyapatite functionalized or not with RGD-containing peptides) on cell adhesion by QCM and to complete these data by using conventional methods such as immunolabelling of vinculin and actin.
Human OsteoProgenitor (HOP) cells were then seeded at a cell density of 12,500 cells/cm2 and cultured on titanium surfaces, functionalized with hydroxyapatite (Ti-HA), type I collagen (Ti-Coll) or with RGD-containing for different times varying from 1 to 3h. Surfaces with adherent cells were then dedicated to quartz crystal resonator experiments. Impedance measurement is also expressed as a percentage of adherent cells to uncoated Ti quartz crystal resonator which was used as control. In parallel, we examine the intracellular distribution of F-actin and vinculin by CLSM for the imaging of focal contact formation. Data obtained by quartz crystal resonator technique revealed that RGD-containing peptides alone, increase HOP cell adhesion in early time period of culture. Moreover, association of RGD-containing peptides with either type I collagen or with HA layers induces an additive effect on HOP cell adhesion compared to Ti-Coll or Ti-HA. By CLSM, both the area of contact focal by cell unit and the cytoskeleton network organization, differed according to the surfaces. Interestingly, association of RGD-containing peptides with HA layers induces an additive effect on focal contact formation on HOP cells compared to Ti-HA alone. These data confirm that RGD peptide effect occurs in the early time of culture, provides a benefit for osteoblast to spread, differentiate and survive.

***Disclosures:* *J. Amedee**, None.*

SA102

***In vivo* Bone Matrix Density Measurements by Water and Fat Suppressed Projection MRI (WASPI).** Y. Wu[1], J. L. Ackerman*[2], H. Cao*[1], T. G. Reese*[2], M. I. Hrovat*[3], M. J. Glimcher[1]. [1]Orthopaedic Surgery, Children's Hospital, Boston, MA, USA, [2]Radiology, Massachusetts General Hospital, Boston, MA, USA, [3]Mirtech, Inc, Brockton, MA, USA.

Bone matrix density plays a significant role in the mechanical and physiological properties of bone substance, as exemplified by the critical role in defining the diagnosis of metabolic bone diseases.
Currently available X-ray based bone densitometry provides only bone mineral density. In conventional MRI, solid bone is not visible due to very short T2 relaxation time.
Water and fat suppressed proton projection MRI (WASPI) has been recently developed to suppress the dominating long T2 signal (water and fat) while preserving the short T2 signal of solid bone and other molecularly immobile substances of the matrix.
A volume transmit/receive MRI coil was built on a Teflon tube (to eliminate proton background signals) to image the ankle of pigs. A very fast crossed-diodes T/R switch and a receiver dead time of 10 micro-s were utilized to detect short T2 signal.
Yorkshire pigs 2-3 months old were scanned with WASPI and conventional gradient echo MRI in a Siemens Trio 3T system while under anesthesia, with the right rear foot inside the coil. Three polymer pellets of different densities were taped on the skin of the foot, serving as calibration phantoms. Fig. 1 shows a coronal view of conventional MRI with slice thickness 3 mm and in plane resolution 1 mm. The muscle, marrow and fat are clearly visible, while the bone is dark and the polymer pellets cannot be seen. Fig. 2 shows a coronal view of WASPI of the same portion of the foot with an isotropic resolution of 2.4 mm. The total 3D imaging time was 27 min. The cortical bone of phalanges and the polymer pellet are bright, while the muscle, marrow and fat signals are suppressed to background levels.

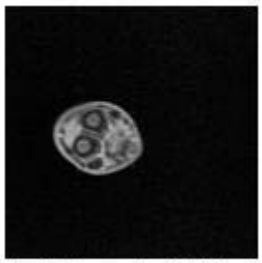


Fig 1. Conventional MRI of the foot of a 2- month old pig. Solid bone matrix and polymer pellets of the calibration phantom do not yield MR signal.

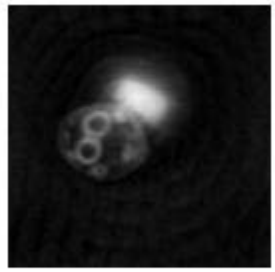


Fig 2. WASPI of the same pig. Cortical bone tissue and the polymer pellets are visualized and the soft tissue signal is suppressed.

This study demonstrates that *in vivo* WASPI measurement is feasible. We showed in a previous *in vitro* study that the WASPI intensity correlates highly with gravimetric and amino acid analysis of matrix density. We therefore anticipate that quantitative bone matrix density measurement by WASPI will be possible.

***Disclosures: Y. Wu**, None.*
This study received funding from: NIH/NIBIB.

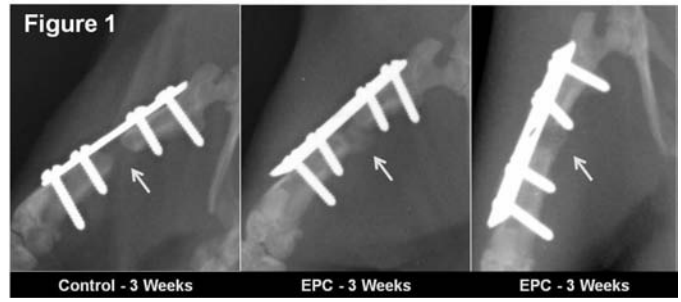
SA103

See Friday Plenary number F103.

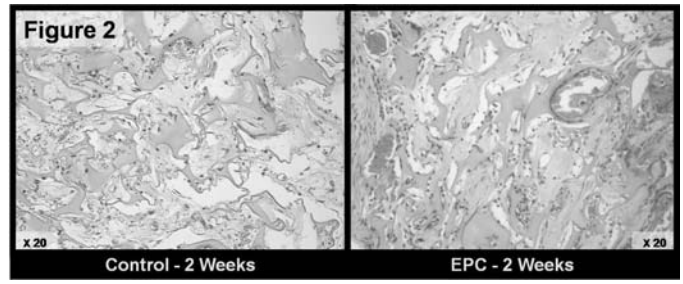
SA104

The Use of Endothelial Progenitor Cells to Promote Bone Healing: Preliminary Results from a Rat Model Study. K. Atesok*, R. Li*, E. H. Schemitsch*. Division of Orthopaedics, St. Michael's Hospital, Toronto, ON, Canada.

Vascular in growth is an essential and critical mechanism in fracture healing. Endothelial Progenitor Cells (EPCs) have been proven to contribute to repair of injured endothelium and formation of new blood vessels.
We hypothesise that local EPC therapy will enhance angiogenesis at the fracture site and this in turn will promote bone healing by increasing osteogenesis and callus formation.
The purpose of this study is to determine the role of EPCs in bone regeneration and to evaluate the effectiveness of local EPC therapy to accelerate fracture repair.
Rat bone marrow EPCs were isolated and cultured for 7 to 10 days. A segmental bone defect (4mm.) was created in rat femur diaphysis and stabilized with mini plate. A gelfoam carrier impregnated with a solution of EPCs ($1x10^6$) was placed into the fracture gap. Control animals were applied only gelfoam with saline but no cells. Ten rats were included in this pilot study. The rats were divided into two groups. Two control and 3 EPC treated rats were included in each group. Animals in Group 1 and Group 2 were sacrificed 2 weeks and 3 weeks after the procedure respectively. Plain radiographs of the operated femur were taken before sacrifice to verify callus formation and the specimens from the fracture gap were collected for histological evaluation.
Radiological evidence of bone regeneration was remarkable in EPC treated animals both in 2 weeks and 3 weeks compared to control animals where no signs of callus formation was observed at the osteotomy site (Fig. 1).



Histological evaluation revealed that the specimens from EPC treated animals had abundant osteoid islands contained predominantly osteoblasts and osteoclasts with more blood vessels in both groups. Conversely, the specimens from control animals were sparsely ossified with markedly less bone cells and vessels (Fig. 2).\



We conclude that the preliminary data from this study encourages the further investigation of EPCs as a potential therapy to promote bone healing.

***Disclosures: K. Atesok**, None.*
This study received funding from: OTC-AIOD.

SA105

Nanotechnological Scaffold with Combination of Prostaglandin E_2 Receptor EP4 Agonist and rhBMP2, Enhances Bone Repair in the Defect of Mouse Calvarium Bone. P. Kamolratanakul*[1], A. Kawamata*[1], Y. Yamamoto*[2], Y. Ezura*[1], K. Akiyoshi*[2], T. Amagasa*[3], M. Noda[1]. [1]Tokyo Medical and Dental University, Molecular Pharmacology,Medical Research Institute, Tokyo, Japan, [2]Tokyo Medical and Dental University, Institute of Biomaterials and Bioengineering, Tokyo, Japan, [3]Tokyo Medical and Dental University, Maxillofacial surgery, Tokyo, Japan.

Prostaglandin E_2 (PGE_2) is known as an anabolic action on bone formation both in Vivo and in Vitro studies; however, side effect of severe diarrhea in animal was reported. The alternative way to work through PGE_2 effect is PGE_2 receptors, which are identified into 4 subtypes. EP4 is the only PGE_2 receptor subtype that was determined in bone and reported to mediate PGE_2-induced anabolic action on bone formation. Nanogel scaffold provides effective drug-trapping and releasing. This study examined weather EP4 with nanogel scaffold carrier, can be performed to be a supplemental treatment for bone reconstruction. In 36 mice , defects were created by implant drill 3.75mm in diameter at parietal bone of mouse calvariae in present or absent of scaffold reconstruction, then after 4 weeks, mice were sacrificed. Treatment with nanogel scaffold in addition with EP4 agonist (EP4A)100µg and/or rhBMP-2 at low dose (0.5 µg), radiographic investigation and CT analysis revealed that treated with EP4A 100µg or BMP 0.5µg , bone formation areas were not significantly different from nanogel-treated group. On the other hand, combination treatment with EP4A 100µg and BMP 0.5µg, bone formation areas were significantly increased about 50% more than non-supplementary nanogel-treated group ($p<0.01$). This data demonstrated that nanogel scaffold-in supplement of EP4A and BMP-2 combination enhances bone formation at defect site of mouse calvariae.

***Disclosures: P. Kamolratanakul**, None.*

SA106

Calcifications in Children with Juvenile Dermatomyositis (JDM) Contain Several Bone Formation Markers. A. L. Urganus*, Y. Zhao, L. M. Pachman. Cellular and Molecular Pathobiology, Children's Memorial Research Center, Chicago, IL, USA.

Previous analysis of JDM calcifications confirmed the presence of osteopontin (OPN), bone sialoprotein (BSP), and osteonectin (ON) (western blot), and Fourier Transform Infrared (FTIR) spectroscopy documented a much higher mineral to matrix ratio than bone (Pachman, 2006). We hypothesize that the mechanism of JDM calcification differs from that of bone formation. The goal of this study was to determine the presence or absence of SIBLING, bone and apoptosis markers in the pathological calcifications. This study was approved by the Children's Memorial Hospital IRB, and age appropriate informed consent

was obtained. Calcification samples surgically removed from 4 different children with JDM with a long duration of JDM symptoms (36.9 ± 48.3 months) were stained for SIBLING members: OPN (full length, N- and C-terminal fragments), BSP, ON, dentin matrix protein 1 (DMP1), the dentin phosphoprotein (DPP) domain of dentin sialophosphoprotein (DSPP), matrix extracellular phosphoglycoprotein (MEPE); bone markers: osteocalcin (OCN), core binding factor alpha 1 (Cbfa1), and alkaline phosphatase (ALP); osteoclast marker: Tartrate resistant acid phosphatase (TRAP); apoptosis marker: poly(ADP-ribose) polymerase (PARP) and mineral regulator: matrix Gla protein (MGP). The following areas of the samples were examined for these markers: the center and periphery of the mineral deposits, adjacent connective tissue and vascular endothelial cells. In all 4 samples, BSP, DPP, DMP1, OPN (full length, N- and C- terminal fragments), and ALP were present within the deposits, connective tissue, and vascular endothelial cells, while MEPE was not detected in any of the samples. OCN and ON were present within mineral deposits and endothelial cells, while Cbfa1 was present in the deposits and connective tissue. PARP was observed within vascular endothelial cells and connective tissue, indicating cell death. MGP was detected in the deposits and endothelial cells. TRAP staining identified multinucleated cells, osteoclasts, which accumulated at the periphery of calcifications. In conclusion, the osteoclastic activity at the surface of calcifications appeared to represent an attempt to resolve these pathological calcifications. Cell death may contribute to the process of calcification. The calcifications share expression of BSP, DPP, DMP1, OPN, ON, OCN, ALP and Cbfa1 with bone, indicating a possible common mechanism between bone formation and calcification in JDM, despite the higher mineral to matrix ratio found in JDM calcifications.

***Disclosures:* *A.L. Urganus**, None.*
This study received funding from: NIH.

SA107

See Friday Plenary number F107.

SA108

Novel Screening System for Bone Matrix Proteins That Control Bone Mineralization. H. Inoue*[1], T. Nakashima*[1], A. Iwamatsu*[2], A. Suematsu*[1], A. Yamaguchi[3], H. Takayanagi[1]. [1]Department of Cell Signaling, Tokyo Medical and Dental University, Tokyo, Japan, [2]Protein Research Network, Inc., Yokohama, Japan, [3]Section of Oral Pathology, Tokyo Medical and Dental University, Tokyo, Japan.

Bone consists of many kinds of matrix proteins, which are considered to be important for bone formation and mineralization by serving as crystal nucleator and regulator of crystal growth. Although many bone matrix proteins have been identified and characterized so far, the detailed molecular function of the matrix proteins in bone mineralization is still unknown. In this study, for elucidating the molecular mechanism of bone mineralization, we have developed new method for screening bone matrix proteins responsible for the mineralization. After bone marrow and connective tissues were removed from mouse tibia and femur, bone matrix proteins were extracted with acetic acid and thereafter with guanidine chloride from these bones. After the bone matrix proteins were separated with SDS-PAGE, the gel was incubated with the phosphate buffer and thereafter with the calcium buffer for the in-gel mineralization. Some matrix proteins induced calcium phosphate formation, which was detected as red band by alizarin red staining. Dissection of the band with the strongest signal and the peptide mass fingerprinting analysis revealed that this band was identical with bone sialoprotein, which was previously reported to induce the nucleation of hydroxyapatite in vitro. This result suggests that our system is very effective for screening of the nucleators or inducers of calcium phosphate formation. Molecular mechanism of mineralization based on the functions of bone matrix proteins which are under investigation will be discussed.

***Disclosures:* *H. Inoue**, None.*

SA109

See Friday Plenary number F109.

SA110

Mineralization Is Related to Collagen Cross-links in Growing Cancellous and Cortical Bone. N. M. B. K. Willems*[1], R. A. Bank*[2], L. Mulder*[3], G. E. J. Langenbach*[3], T. Grünheid*[4], A. Zentner*[4], T. M. G. J. van Eijden*[3]. [1]Depts. of Orthodontics and Functional Anatomy, Academic Centre for Dentistry Amsterdam (ACTA), UvA and VU, Amsterdam, Netherlands, [2]Dept. of Oral Cell Biology, Academic Centre for Dentistry Amsterdam (ACTA), Amsterdam/ TNO Quality of Life, Leiden, Netherlands, [3]Dept. of Functional Anatomy, Academic Centre for Dentistry Amsterdam (ACTA), Amsterdam, Netherlands, [4]Dept. of Orthodontics, Academic Centre for Dentistry Amsterdam (ACTA), Amsterdam, Netherlands.

Introduction: An examinition of the age-related changes in bone mineral and collagen including its cross-links would extend the knowledge of their concomitant development. Moreover, it might improve our understanding of the processes of modeling and remodeling.
Materials and Methods: Thirty-five pigs, aged between 0 and 100 weeks, were used for analysis. Cancellous and cortical bone samples were obtained from areas near the center and near the poles, respectively. The degree of mineralization of bone (DMB) was determined in cancellous and cortical bone using microcomputed tomography. The amount of collagen and its mature cross-links hydroxylysylpyridinoline (HP) and lysylpyridinoline (LP) were quantified using high-performance liquid chromatography. Correlation coefficients were calculated to determine significant age-related changes. Partial correlation coefficients between collagen parameters and DMB were obtained, controlling for age.

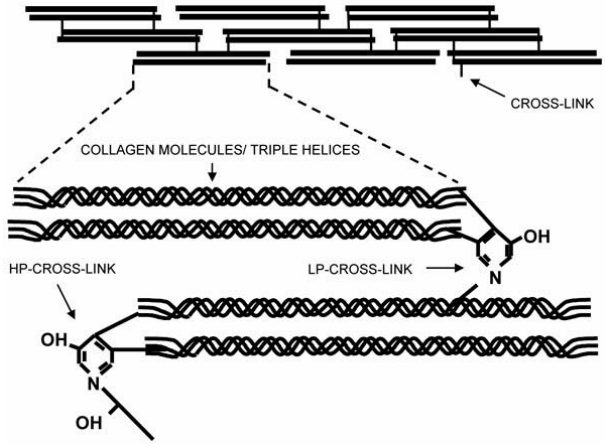


Fig.: Schematic figure of collagen molecules and their mature cross-links HP and LP.
Results: DMB increased with age in cancellous and cortical bone. The amount of collagen increased in cancellous bone, whereas no changes were observed in cortical bone. Moreover, the total number of the HP and LP cross-links decreased in cancellous bone only, because of the decrease found in HP. The number of LP cross-links decreased in cancellous and cortical bone after the age of 10 weeks. DMB and LP correlated significantly in cancellous and cortical bone (r = -0.55 and -0.60 respectively).
Conclusions: Bone collagen and mineralization are related during growth. The high turnover rate in cancellous bone might be responsible for the low number of the mature cross-links HP and LP. The high number of LP cross-links observed in the first weeks suggests LP plays an important role in the onset of bone mineralization.

***Disclosures:* *N.M.B.K. Willems**, None.*
This study received funding from: Inter-University Research School of Dentistry (IOT).

SA111

AC-100 Promotes Cartilage Repair and Sub-Chondral Bone Healing In Surgically Induced Cartilage Defects. C. A. Middleton-Hardie[1], H. Aberman*[2], T. Simon*[2], R. C. Spiro[3], B. Schnegelsberg[1], D. M. Rosen[1]. [1]Research and Development, Acologix, Hayward, CA, USA, [2]Applied Biological Concepts, Los Alamitos, CA, USA, [3]RCS Consulting, Half Moon Bay, CA, USA.

AC-100 showed promising activity in two cartilage defect studies in goats to promote cartilage repair.
The sequence of AC-100 is derived from a central 23-amino acid region of the bone extracellular matrix protein MEPE and is one of the more conserved regions of the molecule. AC-100 has shown specific activity *in vivo* to promote tissue appropriate hard tissue deposition in teeth and in bone.
For the first study, an osteochondral defect (6mm diameter, 6mm deep) was made in the medial femoral condyle of Spanish goats. A cylindrical plug cut from a collagen sponge (CollaPlug; 6mm diameter, 6mm long) was implanted into the lesion site and saturated with the test article (saline; AC-100 2.5, 25mg/application). Post-operative treatments included intra-articular injections (0.5mL) of the appropriate test article into the operated knee joint at 1, 2 and 3 weeks post surgery. All animals were returned to full weight bearing activity post-surgery. The necropsy timepoints were at 3 and 6 months. The joints were evaluated by gross analysis and histology.
There was good correlation between the gross evaluation and the quantitative histological evaluation. Both showed the high dose AC-100 group had better healing outcomes than the saline and low-dose AC-100 groups at both timepoints.
In the second study a full-thickness cartilage defect was created in the medial femoral condyle. In half of the goats the defects underwent a microfracture procedure. The test articles (saline; AC-100 5, 25, 125mg/application) were administered by intra-articular injection (1.5mL) immediately after closing and 1, 2 and 3 weeks post-surgery. All animals were returned to full weight bearing activity post-surgery. In this study a short timeframe of 6 weeks was chosen to investigate early healing processes.
By gross evaluation the AC-100 treated defects showed significant improvements in the amount and quality of repair tissue with a dose-dependent effect (p=0.04 -> p=0.09 vs. saline). Histological evaluation of the edge repair also showed a significant AC-100 effect (p=0.02 -> p=0.07 vs. saline). The AC-100 treatment increased healing with and without microfracture.
As the defects were located in a major weight bearing site the observed improvements seen with AC-100 are very promising. Further studies are underway to investigate the long-term healing effects of AC-100 treated full thickness defects.

***Disclosures:* *C.A. Middleton-Hardie**, Acologix 5.*
This study received funding from: Acologix.

SA112

See Friday Plenary number F112.

SA113

Phosphate Induced Apoptosis in Growth Plate Chondrocytes via a Nitric Oxide and JNK-dependent Pathway. M. Zhong*, Z. Schwartz, B. D. Boyan. Biomedical Engineering, Georgia Institute of Technology, Atlanta, GA, USA.

Hypertrophic chondrocytes in the growth plate exit their development pathway by undergoing apoptosis, in part via the induction of high extracellular phosphate (Pi) concentration involving nitric oxide (NO) production. Recent studies show that high Pi can also initiate apoptosis in resting zone (RC) cells and NO is required. The purpose of the present study was to determine whether the c-Jun N-terminal kinase (JNK), which is associated with apoptosis in many other tissues, contributes to Pi induced apoptosis in RC cells. Rat costochondral RC cells were cultured in DMEM containing 50mM ascorbic acid and 10% FBS. Apoptosis was induced by treatment of confluent cultures with inorganic 7mM phosphate (Pi) for 24h or with the NO donor SNOG. JNK signaling was blocked by incubating the cultures with JNK inhibitor II (EMD Biosciences, Darmstardt, Germany) and NO signaling was blocked using L-NMMA. Apoptosis was assessed as a function of DNA fragmentation ([3H]-thymidine labeled DNA fragments and TUNEL staining) and cell viability using the MTT assay. NO production was determined using a DAN (2,3-diaminonaphthlene) assay. In this study, we found that Pi caused a dose-dependent increase in NO production and a corresponding increase in RC chondrocyte apoptosis, as judged by DNA fragmentation, TUNEL staining and MTT assays. Inhibition of NO production by L-NMMA blocked Pi-dependent apoptosis. Moreover, the NO donor SNOG caused a dose dependent increase in RC chondrocyte apoptosis and inhibition of JNK blocked this effect. In summary, these results show that RC chondrocytes also undergo apoptosis under the induction of Pi, and the pathway is NO-dependent and via JNK. This suggests that one mechanism for preventing premature growth plate closure may be regulation of extracellular Pi production, commensurate with low levels of alkaline phosphatase in the reserve zone.

***Disclosures:* M. Zhong**, *None.*
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SA114

See Friday Plenary number F114.

SA115

Targeted Expression of SOX9 in Hypertrophic Chondrocytes Leads to Enhanced Adipogenic Activity and Spontaneous Osteoarthritis in Transgenic Mice. B. Liang[1], D. Chen*[1], B. Lee[2], G. Zhou[1]. [1]Orthopaedics, Case Western Reserve University, Cleveland, OH, USA, [2]Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA.

The disturbed balance in cartilage metabolism plays an important role in the pathogenesis and progression of osteoarthritis (OA). Transcription factors are key regulators of cartilage metabolism by stimulating chondrogenesis under both physiologic and pathologic conditions. Among them, SOX9 is essential for successive steps of chondrogenesis while RUNX2 is a positive regulator of chondrocytes maturation. RUNX2 also represses adipocytes differentiation and its deficiency in chondrocytes leads to increased adipogenesis. We have previously shown that SOX9 can physically interact with RUNX2 and inhibit its transcriptional activity *in vitro*. To directly investigate SOX9 effects during chondrocyte hypertrophy, a novel *Col10a1-SOX9* transgenic mouse model was generated in which *SOX9* was specifically expressed in hypertrophic chondrocytes driven by a 10kb *Col10a1* promoter. The specific transgene expression in hypertrophic chondrocyte was confirmed by RT-PCR in rib chondrocytes of 6-day-old mice and immunostaining in 10-week-old tibia samples. In monolayer culture of rib hypertrophic chondrocytes isolated from 6-day-old mice, expression of *Col10a1*, a downstream target of Runx2, was decreased by 55% in transgenic mice by quantitative RT-PCR. In comparison with wild-type mice, *Col10a1-SOX9* hypertrophic chondrocytes culture also displayed increased adipogenesis with more lipid droplets accumulation by Oil Red-O staining and higher expression levels of adipogenic genes including *C/EBPα*, *PPARγ2*, *SCD1* and *Glut4*. Additionally, mineralized areas of hypertrophic cartilage were significantly reduced in proximal tibia in 10-week-old *Col10a1-SOX9* transgenic mice. Interestingly, 11-month-old knee joints of *Col10a1-SOX9* transgenic mice but not wild-type littermates developed spontaneous severe osteoarthritis with joint space narrowing, osteophytes formation, and subchondral sclerosis. Moreover, Safranin-O histology revealed severe degradation of articular cartilage and bony outgrowth in 11-month-old transgenic mice but not in wild-type controls. Our results suggest that defective hypertrophic cartilage could contribute to the development of an adverse biomechanical environment and enhance the progression of the articular cartilage deterioration.Therefore, our study not only confirms the direct inhibitory effect of SOX9 on chondrocyte hypertrophy during cartilage formation, it also reveals a potential novel role of SOX9 in osteoarthritis pathogenesis.

***Disclosures:* G. Zhou**, *None.*
This study received funding from: K22 DE015139 NIH/NIDCR.

SA116

See Friday Plenary number F116.

SA117

Effects of Beta-D-glucopyranoside from *Phellodendron Amurensis* on the Production of Inflammatory Cytokines, Growth Factors, Matrixmetalloproteinase, and on Bone Markers in Human Subchondral Osteoarthritic Osteoblasts. D. Kim[1], J. Huh*[2], Y. Baek*[3], D. Choi*[3], D. Park*[3], J. Lee*[3]. [1]Internal Medicine, Kyung Hee University Hospital, SEOUL, Republic of Korea, [2]Oriental Medicine Research Center for Bone & Joint Disease, Kyung Hee University Hospital, Seoul, Republic of Korea, [3]Department of Acupuncture & Moxibustion, College of Oriental Medicine, Kyung Hee University Hospital, Seoul, Republic of Korea.

Objects: Subchondral bone sclerosis is a commom feature of osteoarthritis (OA), but the mechanisms responsible for this condition remain unresolved. we investigate the effects of beta-D-glucopyranoside from *Phellodendron amurensis* on the production of inflammatory cytokines (interleukin (IL)-1α, L-6, and prostaglandin E2 (PGE_2)), growth factors (vascular endothelial growth factor (VEGF) and transforming growth factor-beta (TGF-β)), matrixmetalloproteinase (MMP)/urokinase plasminogen activator (uPA) and bone markers in human subchondral OA Ob.
Methods: We measured the abundance of IL-1α, IL-6, PGE_2, VEGF, TGF-β, MMP-1 and uPA using very sensitive ELISA in conditioned-media of human primary subchondral Ob from normal individuals and osteoarthritic patients. beta-D-glucopyranoside or selective COX-2 inhibitor (NS398) was performed to determine its effect on inflammatory cytokines, growth factors, MMP/uPA. The expression of bone cell markers was also determined.
Results: OA Ob produced all these factors with greater variability than normal cells. Interestingly, the production of IL-6, PGE_2, VEGF and MMP-1/uPA by OA Ob separated patients into two subgroups, those whose Ob produced levels comparable to normal (low producers) and those whose Ob produced higher levels (high producers). In those cells classified as high producers, IL-6, PGE_2, VEGF and MMP-1/uPA levels were increased two- to three-fold and five- to six-fold, respectively, compared with normal. In contrast, we found that IL-1α and TGF-β levels were similar in normal and all osteoarthritic Ob. beta-D-glucopyranoside significantly reduced IL-6, PGE_2, VEGF and MMP-1/uPA levels in all OA Ob. In contrast, using NS398 did not reduce VEGF and MMP-1/uPA levels in either group. The evaluation of alkaline phosphatase activity, osteocalcin release and collagen type I activity increased by beta-D-glucopyranoside in OA Ob regardless to which subgroup they were assigned.
Conclusion: These results suggest that beta-D-glucopyranoside may contribute to bone remodeling through inhibition of inflammation and MMP/uPA system in subchondral OA Ob, ultimately leading to treatment of abnormal bone sclerosis in OA.

***Disclosures:* D. Kim**, *None.*
This study received funding from: Grant of the Oriental Medicine R&D Project(B030008), Ministry of Health&Welfare, Republic of Korea.

SA118

See Friday Plenary number F118.

SA119

Vinculin Is Involved in Hypertrophic Differentiation Through Raf/MEK/ERK Pathway in Chondrocytic ATDC5 Cells. T. Koshimizu*[1], K. Tachikawa*[1], K. Ozono[2], T. Michigami[1]. [1]Department of Bone and Mineral Research, Osaka Medical Center and Research Institute for Maternal and Child Health, Osaka, Japan, [2]Department of Pediatrics, Osaka University Graduate School of Medicine, Osaka, Japan.

Vinculin occurs in multimolecular complexes that function in adhesion and/or signaling between the extracellular milieu and the cell, via integrins and cadherins. Although vinculin-knockout mouse was reported to exhibit impaired limb development, the roles of vinculin in chondrogenesis remain unclear. Gene-trap mutagenesis is based on the idea that the random insertion of a trapping vector may disturb the function of inserted genes. We applied this method in ATDC5, a cell model of chondrocyte differentiation, and isolated a clone where vinculin gene is trapped. In this clone named #4-17, a truncated vinculin lacking paxillin-binding domain was produced from the trapped allele. Phosphorylation of FAK and ERK1/2 induced by adhesion to extracellular cell matrix (ECM) was markedly enhanced in #4-17 compared with parental cells, which is consistent with previous reports in vinculin-null fibroblasts. The expression of vinculin in parental ATDC5 was gradually elevated during the chondrocytic differentiation, and immunostaining for vinculin of tibial growth plates from wild-type mice demonstrated the parallel results. Thus, we investigated the roles of vinculin in chondrocyte differentiation utilizing the clone #4-17. When cultured in differentiation medium, #4-17 exhibited impaired nodule formation and less accumulation of cartilaginous matrices. The expression of *Col2a1* and *PTHrP* was detected in #4-17, but was diminished earlier than the parental ATDC5 cells. The expression of *Col10a1* was also markedly reduced in #4-17. Interestingly, the expression of SOX9 was retained. Then, we examined the effect of the trapping of vinculin gene on signal transduction during the chondrocyte differentiation. Since Raf/MEK/ERK pathway is reported to play a critical role in chondrocyte differentiation, we investigated the

phosphorylation of c-Raf and ERK1/2. In parental ATDC5 cells, phosphorylation of c-Raf and ERK1/2 was increased during the chondrocytic differentiation. On the other hand, phosphorylation of c-Raf and ERK1/2 was rather declined in #4-17 when cultured in the differentiation. The result makes a striking contrast with the enhanced phosphorylation of ERK1/2 induced by adhesion to ECM. These data suggest that vinculin plays an important role in hypertrophic differentiation of chondrocytes, and that Raf/MEK/ERK pathway might be involved in the regulation of hypertrophic differentiation by vinculin.

Disclosures: ***T. Koshimizu****, None.*

SA120

Mouse-to-Human Strategy to Identify Bone Strength QTL Genes. C. L. Ackert-Bicknell[1], B. Paigen*[1], W. G. Beamer[1], C. J. Rosen[1], S. Demissie*[2], L. A. Cupples*[2], Y. H. Hsu[3], D. P. Kiel[3], D. Karasik[3]. [1]The Jackson Laboratory, Bar Harbor, ME, USA, [2]Biostatistics, BU Sch Public Health, Boston, MA, USA, [3]IFAR, Hebrew SeniorLife, Boston, MA, USA.

Murine models have proven to be very useful for the identification of candidate genes underlying complex traits. A large number of resources are now available to allow for translatability of gene prediction in mice to human populations. We have identified QTLs for Bone Mineral Density (BMD) on proximal Chr 15 in mice in two separate F2 mapping crosses: B6xC3H (peak at 11.9 cM) and B6xNZW (peak at 17 cM). The 95% confidence interval (CI) for the B6xC3H QTL peak extended from 4.0 cM to 28.0 cM (10 Mb to 59.96 Mb) and encompassed the CI for the B6xNZW QTL peak. This region is predicted to contain greater than 270 genes. Using block haplotyping we identified 18 distinct haplotype blocks covering 12.4 Mb. In total 52 genes were found to be either completely or partially included in these haplotype blocks. Also, a QTL for BMD was reported in the literature in a B6xDBA cross with a peak at 10 cM. When DBA was added to the haplotype analysis, we were able to exclude 22 genes as candidates. The largest of these blocks was located immediately distal to the *Trps1* gene. This gene encodes a specific zinc finger protein that is a putative transcription factor. The human *TRPS1* gene has been shown to be involved in trichorhinophalangeal syndrome (also known as Langer-Giedion syndrome with exostoses). To translate the mouse finding to humans, we performed a genetic association study of polymorphisms (SNPs) in the vicinity of the *TRPS1* in a subset of unrelated individuals from the Framingham Offspring cohort. This cohort consisted of 793 women and 700 men (age 61.3 ± 9.1 yrs), for whiom both DNA and bone phenotypes (DXA measurements of the hip and spine and/or heel QUS) were available. We genotyped 22 tagging SNPs in the region on chr. 8q24, covering 93% of the variance in *TRPS1* gene, using the SNPlex™ and ABI TaqMan platforms. We assessed the association between SNPs and bone phenotypes using sex-specific multiple linear regression, adjusted for age, BMI, height, smoking, and estrogen status (in women). To correct for the numerous statistical tests in our analysis, we performed permutation tests. After adjustment for multiple testing (permuted 10,000 times), 2 SNPs near the *TRPS1* were identified to be significantly associated (p values ≤ 0.0001) with the bone traits. One SNP (rs720928, associated with femoral BMD and heel QUS in men at p<0.0005) is located distal to the *TRPS1* gene and this finding corresponds to our haplotype analysis performed in mice. We are currently exploring other genes in this haplotype block. This comparative genomic approach using mouse models may be an efficient strategy to identify new candidate genes associated with bone phenotypes in humans.

Disclosures: ***D. Karasik****, None.*
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SA121

Intracellular PTHR2 Alters Cell Proliferation and Pattern of Gene Expression while Antagonizing PTHR1 Signalling in Chondrocytic CFK2 Cells. D. K. Panda*, D. Goltzman, A. C. Karaplis. Experimental Medicine, McGill University, Montreal, QC, Canada.

TIP39 and PTHR2 are extended members of parathyroid hormone ligand and receptor family. Both the ligand (TIP39) and receptor (PTHR2) are expressed in the growth plate, the former in well differentiated hypertrophic chondrocytic cells while the latter in cells of the periarticular cartilage. This expression pattern is completely opposite to that of PTHrP and PTHR1 in the developing growth plate.
We have observed that upon TIP39 stimulation, PTHR2 expressed in CFK2 chondrocytic cells undergoes endocytosis and accumulates in the perinuclear / nuclear compartment. A bipartite nuclear localization motif is present in the C-terminal region of the receptor which also contains more than one nuclear export signature. These findings prompted us to hypothesize that PTHR2 may function intracellularly, in part at the level of the nucleus and antagonize PTHrP/PTHR1 signalling. To explore the potential for intracellular action by PTHR2, we deleted the signal sequence from the N-terminal portion of the receptor so that only the mature protein would be expressed. We then stably transfected chondrocytic CFK2 cells with this construct (ΔSSPTHR2). In the absence of TIP39, expression of ΔSSPTHR2 alone, decreased proliferation and suppressed 3H thymidine incorporation in CFK2 cells. This parallels the effects of the full length receptor on cellular proliferation following TIP39 stimulation. In addition, mRNA microarray analysis was performed to get some insight into the altered gene expression pattern between in CFK2/ΔSSPTHR2 compared to CFK2/vector cells. Expression of the transcription factor Sox9 as well as matrix proteins such as type II collagen and matrix gla protein (Mgp) were highly suppressed by ΔSSPTHR2 in CFK2 cells, as observed following treatment of full-length PTHR2 transfected CFK2 cells with TIP39. The microarray data was reconfirmed by semi-quantitative RT-PCR analysis. When 1 kb of the mouse Mgp promoter inserted in a luciferase reporter system was cotransfected with ΔSSPTHR2, the activity of the reporter plasmid was reduced three- to five-fold. Interestingly, MGP expression was up-regulated by PTHrP/PTHR1 signalling at the transcriptional level. To resolve this conundrum, we expressed a constitutively active form of PTHR1(H410P) along with leaderless form of PTHR2. When, PTHR1(H410R) was co-expressed along with ΔSSPTHR2, Mgp expression was restored to levels comparable to vector transfected CFK2 cells.
In summary our results suggest, the leaderless form of PTHR2 functioning intracellularly recapitulates the proliferative and gene expression patterns of the full length receptor following TIP39 stimulation. Moreover, PTHR1 and PTHR2 may display antagonistic signalling in chondrocytes.

Disclosures: ***D.K. Panda****, None.*

SA122

See Friday Plenary number F122.

SA123

Hypothyroidism Is Not Deleterious to the Skeleton During Early Fetal Development. L. P. Capelo*[1], E. H. Beber*[2], T. M. T. Zorn*[1], S. A. Huang*[3], A. C. Bianco*[4], C. H. A. Gouveia[2]. [1]Departmento of Cell and Developmental Biology, Instute of Biomedical Sciences, University of Sao Paulo, Sao Paulo, Brazil, [2]Departmento of Anatomy, Instute of Biomedical Sciences, University of Sao Paulo, Sao Paulo, Brazil, [3]Division of Endocrinology, Children's Hospital Boston, Boston, MA, USA, [4]Division of Endocrinology, Diabetes and Hypertension, Brigham and Woman's Hospital, Boston, MA, USA.

Thyroid hormone (TH) has a key role on post-natal bone development, while its relevance during fetal bone development is less clear. To investigate this, we induced a hypothyroid state and analyzed the skeleton during pre- and post-natal development of mice. We also examined the mRNA expression of the monocarboxilate transporter 8 (MCT8), a plasma membrane transporter that mediates the cellular influx and/or efflux of TH, and the expression of the iodothyronine deiodinases type II and III (D2 and D3). D2 is an activating enzyme that converts thyroxine (T4) into its active form, triiodothyronine (T3), while D3 inactivates T4 and T3. Hypothyroidism (HYPO) was induced pharmacologically during gestation and nursing. The fetuses and litters were harvested at embryonic days (E) 14.5, 16.5 and 18.5 and at post-natal days (P) 4, 7, 14, 21 and 35. Histological analysis of the distal epiphyseal growth plate (EGP) of the femur and vertebra showed that HYPO barely affected bone development up to E16.5. Only at E18.5, the length and area of the proliferative and hypertrophic zones, the number of chondrocytes per proliferative column and the number of hypertrophic chondrocytes were reduced in the EGPs of HYPO fetuses. Femoral D3 mRNA was detected at E14.5 and its expression decreased markedly (~10-fold) at E18.5, and even more at P14. In contrast, femoral D2 mRNA expression increased significantly by E18.5 and markedly (~2.5-fold) by P14. At E18.5, D2 activity was increased while D3 activity was diminished in the femur of HYPO fetuses (~2 fold). Like D3, the femoral mRNA expression of MCT8 decreased significantly from E14.5 to P35 (~5-fold) and was up-regulated in the femur of HYPO animals during post-natal development. In conclusion, the absence of a HYPO-induced bone phenotype during early fetal development suggests that TH signaling in bone is kept to a minimum at this stage. In addition, the reciprocal expression pattern of D2 and D3 during skeletal development suggests that these enzymes may be responsible for keeping low levels of TH in the bone for the proper early fetal bone development. However, the MCT8 expression suggests that a minimum TH influx may be necessary during the same period. In summary, D2 and D3 are likely to have an important role during pre-natal development of the skeleton, while MCT8 may be important to modulate TH effects on bone, mostly during post-natal development.

Disclosures: ***L.P. Capelo****, None.*
This study received funding from: FAPESP

SA124

Functionalization of a PLGA/PEG-PLA Composite Electrospun Scaffold with rhBMP-2 Plasmid DNA for Bone Regeneration. X. Zhao*[1], B. Hsiao*[2], B. Chu*[2], M. Hadjiargyrou[1]. [1]Biomedical Engineering, SUNY, Stony Brook, Stony Brook, NY, USA, [2]Chemistry, SUNY, Stony Brook, Stony Brook, NY, USA.

Previously, our laboratory reported on the fabrication of DNA based nanofibrous and biodegradable electrospun scaffolds designed to serve as gene delivery systems for *in vivo* tissue engineering applications. In the current study, using electrospinning, we generated two scaffolds, one using a combination of 30% (w/w) poly lactide- co-glycolide (PLGA) and 3% (w/w) diblock copolymer (PEG-PLA) and by incorporating 0.025% (w/w) rhBMP2 plasmid (~1mg), and the other without any DNA. The scaffolds generated measured 15cm (width) x 30cm (length) x 100µm (thickness). Scanning electron microscopy revealed that both non-woven scaffolds looked indistinguishable and had a range of fiber diameters between 500 nm - 1.5 µm. Subsequent DNA release studies using 1.5 x 1 cm sections of the rhBMP2 DNA based scaffold showed an ~60% of DNA (in TE buffer) release in the first 24 hrs and then a steep decrease over time. Collectively, ~70% of plasmid DNA was released over 5 days, corresponding to about 2µg. Gel electrophoresis confirmed that the released plasmid was structurally intact. We are now conducting experiments in order to test the released rhBMP2 plasmid's bioactivity, by transfecting pre-osteoblastic MC3T3 cells with both the original rhBMP2 plasmid and the released DNA to see if they can enhance differentiation and mineralization. In addition, we are also carrying out *in vitro* studies with MC3T3 cells plated on top of the two types of scaffolds, again to test the effects on differentiation and mineralization. Lastly, we are now starting *in vivo* experiments using the tibial drill hole (critical size defect) model in order to assess the scaffolds capability of enhancing skeletal regeneration. Overall, we hope that the rhBMP2 plasmid DNA based scaffold will be a viable gene delivery system and have the potential to deliver therapeutic rhBMP2 plasmid DNA to stimulate tissue regeneration.

***Disclosures: M. Hadjiargyrou**, None.*
This study received funding from: New York Center for Advanced Technology/STAR Inc.

SA125

Strong and Extensive Epistatic Interactions Affect Bone Traits in a Mouse Reciprocal Intercross of HcB/8 and HcB/23. N. Saless[1], S. J. Litscher*[1], R. S. Kattapuram*[1], M. J. Houlihan*[1], T. K. O'Neil*[1], S. Sudhakaran*[1], K. G. Abdul Raheem*[1], S. Olson*[1], P. Demant*[2], R. D. Blank[1]. [1]Medicine/Endocrinology, University of Wisconsin and Madison VAMC, Madison, WI, USA, [2]Genetics, Roswell Park Cancer Institute, Buffalo, NY, USA.

Linkage studies have identified quantitative trait loci (QTLs) underlying bone mineral density, bone geometry, and bone biomechanics in multiple organisms. Less progress, however, has been made in identifying the genes responsible for these QTLs. Moreover, the mapped QTLs account for only a minority of the heritable component of the measured bone properties. We hypothesize that much of the "missing" genetic contribution to bone properties arises from epistatic interactions between genes. To explore this possibility, we performed an epistasis analysis in a 603 animal, reciprocal F2 intercross of the recombinant congenic strains HcB/8 and HcB/23 that was analyzed for femoral biomechanical performance and geometry.
Animals were maintained under standard husbandry conditions and sacrificed at 17 weeks. Genotyping was done using 39 microsatellite markers spanning the approximately 300 centiMorgans over which the parental strains are known to be genetically informative. The cross includes informative regions on chromosomes 1, 2, 3, 4, 6, 9, 10, 15, 17, and 19. In single locus genome scans reported previously, we found QTLs for bone related traits on chromosomes 1, 2, 3, 4, 6, and 10. We performed the epistasis analysis with the R-QTL linkage package.
Nearly every possible pair of chromosomes yielded a significant epistatic interaction for at least 1 trait. The most striking interactions involve chromosomes 1 and and 3. On chromosome 1, there are significant interactions between distinct loci on the chromosome, as well as with all the other informative chromosomes. On chromosome 3, the same pattern exists, with a significant intrachromosomal interaction as well as epistatic interactions with all the other informative chromosomes. The LOD scores for the full models, including epistasis, range from 8 to 18.
Because only approximately ¼ of the genome segregates between pairs of recombinant congenic strains, they are particularly useful for studying epistasis, as the number of potential statistical tests is reduced approximately 16-fold in comparison to an intercross between ordinary inbred strains. This increases the power to detect epistasis when present. Studying epistasis is important not only because it accounts for previously unmeasured genetic contributions to bone properties, but because interactions provide additional clues for identifying the genes underlying QTLs.

***Disclosures: N. Saless**, None.*
This study received funding from: Veterans Administration.

SA126

A Bone Defect Model in Mouse Femur to Challenge Biomaterials, Cells and Molecules for Bone Repair. J. Fricain*[1], L. Monfoulet*[1], B. Rabier*[1], L. Malaval*[2], J. Amedee[1], O. Chassande*[1]. [1]INSERM U577, Bordeaux, France, [2]INSERM U890, Bordeaux, France.

Bone loss treatment is still a challenge for orthopaedic and oral surgeons. Experimental models are useful to better understand natural bone repair and to investigate the function of therapeutic molecules in bone repair. Mice have been used for skeletal research and the defect models used have interested the femur diaphysis to study cortical reparation or metaphysis to study cancellous bone cicatrisation. The aim of this study is to demonstrate the influence of the size of a mice femur metaphyseal defect on density and volume of cancellous and cortical newly bone formed and to create a critical size defect valuable to measure the effect of molecules or biomaterials on bone repair. Holes of 0.9 and 1.6 mm were performed respectively in the right and left femur of C57Bl6/J male mice (14 weeks old) and the femur were retrieved at 14, 28, 56 and 90 days, fixed in 4% paraformaldehyde for 24 hours at 4Â°C, then transferred into 70% ethanol and kept at 4Â°C. On one hand, femurs were scanned in a Explore Locus SP X-Ray microcomputerized tomography device (GE). On the other hand, femurs were decalcified in 0.5M EDTA for 3 days, then transferred in 70% ethanol, embedded in paraffin and 3 Î¼m thick sections were stained with HES. Qualitative analysis of bone repair revealed that 14 days after surgery, fibrous tissue was mainly observed in 1.6mm defect while primary cancelous bone was present in the inner surface of 0.9mm defects. 28 days after surgery, 0.9 mm defects, were completely filled with trabecular bone while fibrous tissue was still present in 1.6 mm defects. The cortical edge of the 0.9 mm diameter defect was partially closed with dense mineralized tissue but remained completely opened in 1.6mm diameter defects. At 56 days, no marked evolution was observed in 0.9 mm defects as compared with 28 days. In 1.6 mm defects, fibrous tissue was still present in the external part of the lesion whereas trabecular like structures invaded most of the defect volume. Similar results were observed at 90 days. Quantitative analysis of cancellous bone formation showed that bone volume fraction (BVF) in the 0.9 mm defect was more important than in 1,6mm defect, due to more important trabecular number while thickness and density (BMD) were similar. At 28, 56 and 90 days no statistical difference was observed between the two size defects. For quantitative analysis of cortical bone formation, in 1.6 mm diameter defects cortical, BVF was lower than in 0.9 mm defects at 28 days, and remained very low until 90 days, reflecting failure to close the perforation. In conclusion, the 1.6mm defect in mice is a cortical critical defect that could be used to test the action of molecules, cells or biomaterials on bone repair.

***Disclosures: J. Amedee**, None.*

SA127

See Friday Plenary number F127.

SA128

Micro-CT Abnormalities in the th3 Mouse Model of Thalassemia Start at a Young Age and Are Associated with Decreased Bone Turnover. M. G. Vogiatzi[1], J. Tsay*[1], J. Racchumi*[1], S. Rivella*[1], R. W. Grady*[1], S. B. Doty[2], P. J. Giardina*[1], A. L. Bosley[2]. [1]Pediatrics, Weill Cornell Medical College, New York, NY, USA, [2]Hospital for Special Surgery, New York, NY, USA.

Low bone mass and fractures occur frequently in patients with beta Thalassemia Intermedia (TI), a type of congenital hemolytic anemia characterized by decreased synthesis of the beta globin chains of hemoglobin. To better understand the bone changes in TI, we studied the th3/+ mouse model of this disease. Th3/+ mice bear a heterozygous deletion of the mouse beta globin genes and, similar to humans with TI, manifest mild to moderate anemia and iron overload as well as splenomegaly.
Th3/+ mice and age-matched WT controls (6 mice per group) were studied at age 2, 4, 6 and 9 mo by micro-CT (both femurs and vertebrae) followed by mechanical testing (three-point bending). We also performed immunostaining for procollagen I and TRAP, histomorphometry for mineral apposition rate (MAR), RT-PCR for osteoprotegerin (OPG), RANKL and cathepsin K.
Compared to WT animals: i) Micro-CT of trabecular bone (L5 vertebra) showed decreased bone volume fraction, decreased number of trabeculae and increased trabecular separation in the th3/+ mouse at all ages ($p<0.05$). ii) Cortical bone at femur mid-diaphysis showed increased marrow area and decreased elipticity (Imax/Imin) of the th3/+ animals ($p<0.05$ at all ages). iii) Micro-CT parameters in the th3/+ mice did not worsen with age. iv)Th3/+animals exhibited decreased MAR (1.4 ± 0.3 vs. 2 ± 0.5, th3/+ vs. WT, $p=0.05$, age 4mo) and decreased expression of cathepsin K, OPG and RANKL ($p<0.01$ at all ages). The number of osteoclasts and % bone surface covered by osteoblasts were the same in both th3/+ mice and controls. iv) Biomechanics showed reduced maximum load (20 ± 3 vs. 15.5 ± 2.6, th3/+ vs. WT, $p=0.01$), maximum moment (35.6 ± 5 vs. 27 ± 4.5, th3/+ vs. WT, $p=0.01$) and structural stiffness (106 ± 19 vs. 134 ± 23 th3/+ vs. WT, $p=0.05$) in the th3/+mice.
In conclusion, the th3/+ mouse model of TI manifests changes in bone micro-architecture, geometry and mechanical properties reminiscent of those in humans. Micro-CT abnormalities are present by age 2 mo and do not worsen or improve with age, suggesting problems with both bone accrual and remodeling. In fact, bone changes in this model are associated with decreased bone turnover.

***Disclosures: M.G. Vogiatzi**, None.*
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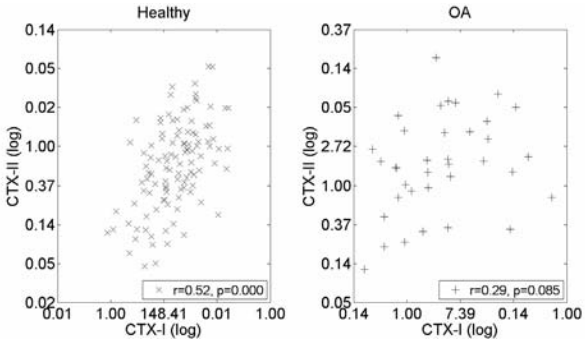
SA129

Longitudinal Relationships between Cartilage and Bone Turnover in Osteoarthritis. E. B. Dam*[1], M. A. Karsdal[2], I. Byrjalsen[2], A. B. Jensen*[2], C. Christiansen[2]. [1]Imaging, Nordic Bioscience, Herlev, Denmark, [2]Nordic Bioscience, Herlev, Denmark.

Purpose: The pathogenesis of osteoarthritis (OA) is speculated to involve interaction patterns of bone and cartilage turnover. We investigated cross-sectional and longitudinal relationships between markers for cartilage and bone turnover in healthy subjects and subjects with OA symptoms. The focus was cartilage loss and correlations between bone and cartilage markers.
Methods: The 21-month study included 159 subjects prospectively selected as representative for the general population. To ensure a homogeneous population, only those over 30 years at were included, resulting in 145 subjects with age 58±13, BMI 26±4, 48% female, and 25% with OA (Kellgren and Lawrence >1). MRI scans were acquired using a Turbo 3D T1 sequence on a 0.18T Esaote scanner and total medial tibial and femoral cartilage volume was quantified automatically in a computerized framework. Bone resorption was measured by the biochemical marker serum CTX-I (C-terminal telopeptide of collagen type I) and cartilage degradation by urine CTX-II.
Results: At baseline (BL) CTX-I and CTX-II were linearly correlated in the healthy population (r=0.52, p<0.001) but only borderline for the OA subjects (r=0.29, p=0.09).



Inspection of the OA subjects showed that elevated CTX-II scores at BL predicted longitudinal cartilage loss. Specifically, the odds ratio (OR) for increased cartilage loss was 4.0 - comparing highest and lowest tertiles of CTX-II (p<0.01). The same OR for CTX-I was 1.4 (not significant). There was a correlation between BL CTX-II scores and follow-up (FU) CTX-I (r=0.23, p<0.01). Contrarily, there was no correlation between BL CTX-I and FU CTX-II (r=0.08, p=0.4). All results persisted after correction for gender and age.
Conclusion: The results support a coupling between cartilage and bone turnover in healthy individuals that however is misbalanced in OA patients. In healthy individuals, a balanced cartilage and bone turnover may be of major importance for joint health. This emphasizes a potential need for OA treatments that restore the bone/cartilage metabolic balance. By comparison of BL and FU biomarker levels, it could be hypothesized that at least a specific stage of cartilage breakdown (elevated CTX-II at BL) is preceding bone remodeling (elevated CTX-I at FU) in OA - possibly a piece for the complex puzzle of causal relationships between cartilage and bone breakdown.

***Disclosures: M.A. Karsdal**, None.*
This study received funding from: Nordic Bioscience.

SA130

Apc-mediated Control of β-catenin Is Essential for Both Chondrogenic and Osteogenic Differentiation of Skeletal Precursors. R. L. Miclea*[1], E. C. Robanus-Maandag*[2], C. A. J. Bosch*[2], T. Kobayashi[3], H. M. Kronenberg[3], G. Rawadi*[4], C. W. G. M. Löwik[5], R. Fodde*[6], J. M. Wit*[1], M. Karperien[7]. [1]Pediatrics, Leiden University Medical Centre, Leiden, Netherlands, [2]Human Genetics, Leiden University Medical Centre, Leiden, Netherlands, [3]Endocrine Unit, Massachusetts General Hospital, Boston, MA, USA, [4]Galapagos, Romainville, France, [5]Endocrinology, Leiden University Medical Centre, Leiden, Netherlands, [6]Pathology, Josephine Nefkens Institute, Erasmus MC, Rotterdam, Netherlands, [7]Tissue Regeneration, Institute for Biomedical Technology, Twente University, Enschede, Netherlands.

During endochondral bone formation, levels of transduced canonical Wnt/β-catenin signaling determine lineage commitment of skeletal precursor cells: high levels induce osteoblast differentiation, whereas low levels lead to chondrocyte differentiation. Whether Adenomatous polyposis coli (Apc), the key intracellular gate-keeper controlling β-catenin turnover, is involved in this process is currently unclear.
To better understand the mechanisms that modulate β-catenin levels in skeletal precursor cells we generated conditional knockout mice lacking functional *Apc* in *Col2a1*-expressing cells.
Conditional heterozygous and homozygous *Apc* mutants were monitored for the development of an abnormal phenotype. Heterozygous Col2a1-*Cre*-mediated *Apc* inactivation did not interfere with embryonic skeletal development, postnatal growth or bone mass acquisition up to 12 weeks of age. In marked contrast, conditional homozygous mutant Col2a1-*Cre*;*Apc*$^{15lox/15lox}$ mice died perinatally showing greatly impaired skeletogenesis. The skeletal defects were already present at E12.5. All endochondral bones were misshaped and lacked structural integrity. Lack of functional Apc resulted in a pleiotropic skeletal cell phenotype. The vast majority of the skeletal precursor cells lacking *Apc* failed to differentiate and retained a mesenchymal-like spindle shape morphology. However, skeletal precursor cells in the proximal ribs were able to escape the noxious effect of functional loss of Apc resulting in formation of highly active osteoblasts. Inactivation of Apc in chondrocytes was associated with dedifferentiation of these cells.
We conclude that Apc plays a crucial role in modulating the β-catenin level during mouse skeletogenesis in a spatio-temporal regulated manner. In skeletal precursor cells, Apc is required for differentiation into both chondrocytes and osteoblasts. In chondrocytes, Apc is essential to maintain the chondrocytic phenotype and enable maturation.

***Disclosures: R.L. Miclea**, None.*
This study received funding from: IPSEN, European Society for Pediatric Endocrinology (ESPE), Human Growth Foundation.

SA131

See Friday Plenary number F131.

SA132

Differences in Femoral Bone Structure Between Juvenile Non-transgenic and Transgenic Rabbits with the WAP-hFVIII Gene Construct. M. Martiniakova*[1], R. Omelka[1], B. Grosskopf*[2], K. Dammers*[3], M. Bauerova*[1], P. Chrenek*[1]. [1]Constantine the Philosopher University, Nitra, Slovakia, [2]Georg-August University, Göttingen, Germany, [3]Konyang University, Nonsan, Republic of Korea.

Transgenic rabbits represent an alternative way of producing recombinant human proteins and enzymes as well as serving as an appropriate animal model for different human diseases. Transgenic technology, however, can also produce possible changes in tissues other than target ones. In a previous study we identified a new type of bone tissue - fibrolamellar tissue - in 2.5 month-old transgenic rabbits carrying the WAP-hFVIII gene construct (AHE 35: 310-315, 2006). This tissue has not been identified in rabbits even in any ontogenetic stage up to date. In the present study femoral bone structure in younger (1 and 2 month-old) non-transgenic and transgenic rabbits were compared with the focus on finding the age at which the differences first appear. Altogether, 28 clinically healthy rabbits (14 transgenic and 14 non-transgenic) were analyzed. Femoral bone microstructure was evaluated in terms of qualitative and quantitative histological characteristics. In an effort to better understand the changes, concentrations of 9 bone-related mineral elements, solids and total mineral content in the femora, as well as a rate of aneuploidy in bone marrow cells were also analyzed. We identified fibrolamellar bone tissue again only in the transgenic rabbits (both 1 and 2 month-old ones). Measured values for area and perimeter of the Haversian canals and minimum diameter of the primary osteons' vascular canals were higher in the transgenic individuals (P<0.05). On the other hand, minimum diameter of the secondary osteons was higher in non-transgenic rabbits (P<0.05). We observed lower concentrations of Ca, P, K, solids and total mineral content in the femora of transgenic rabbits. A significant difference was observed for concentration of Ca (P<0.05). A significantly higher rate of aneuploidy cells from bone marrow in the transgenic individuals was also identified (P<0.05). Our results indicate evident changes in the structure of the femur in 1 and 2 month-old transgenic rabbits which result especially in a better blood supply and a slightly reduced mineralization process. For possible applications of the results, it is necessary to verify them in successive ontogenetic stages of rabbits (mainly in new-born non-transgenic and transgenic individuals) and to explain the findings on molecular level. In the future, a genetic analysis followed by a histological one in this unique model could reveal new information about the genetic contribution to the microstructure of analyzed bones. This study was supported by the grant KEGA 3/4032/06.

***Disclosures: M. Martiniakova**, None.*

SA133

Gender-Specific Changes in Bone Microstructure of Mice Lacking Ribosomal Protein L29/HIP: An FT-IR Imaging Analysis. L. G. Sloofman*[1], L. Spevak*[2], A. L. Boskey[2], M. C. Farach-Carson[1], C. B. Kirn-Safran*[1]. [1]Biological Sciences, University of Delaware, Newark, DE, USA, [2]Hospital for Special Surgery, Cornell University-Weill Medical College, New York, NY, USA.

Adult HIP/RPL29-deficient mice display a short stature phenotype and were shown recently to exhibit increased bone fragility due to altered matrix protein synthesis rates (1). The purpose of this study is to provide insights into structural molecular changes that occur in HIP/RPL29-null bones versus wild type (WT) controls. To measure differences in bone properties, we examined the Fourier transform infrared (FT-IR) spectroscopic profiles of hard resin-embedded tibia sections in null mutants and WT littermates. We found that the mineral-to-matrix ratios were increased in trabecular bone and growth plate cartilage of null male tibias compared to gender-matched WT at 12 weeks. This data is consistent with previous studies performed in 24 week-old femurs that indicated that preservation of cortical thickness is associated with increased bone mineral density only in null males (1). Although female null bones did not display significant changes in their mineral-to-matrix ratios relative to gender- and aged-matched WT, their growth plate cartilage displayed a significant decrease in carbonate-to-mineral ratio. In addition, collagen maturity was severely reduced in both growth plate cartilage and trabecular bone as shown by a decrease in collagen cross-links. In contrast, our studies did not indicate significant differences in crystal growth between null and WT in either males or females. Our findings confirmed previous data that pointed to gender-specific effect of the genotype on bone material properties. We propose that in null females, ribosomal insufficiency has more pronounced consequences on the relative distribution of organic versus inorganic phases rather than absolute quantity of mineral than in null males. Ongoing work uses the HIP/RPL29 mouse model to understand the mechanisms related to protein translation that underly gender related bone fragility and fracture risk.

1. Oristian, D.S., Sloofman, L.G., Zhou, X., Wang L, Farach-Carson, M.C., and Kirn-Safran, C.B. (in press) *J. Orthop. Res.*

***Disclosures:* *C.B. Kirn-Safran**, None.*

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SA134

Bone Marrow Stromal Cell-derived Extracellular Matrix Promotes Mesenchymal Stem Cell Motility. J. Ling*[1], Y. Lai*[2], C. M. Skinner*[2], X. Chen[2]. [1]Bioengineering Section, Southwest Research Institute, San Antonio, TX, USA, [2]Restorative Dentistry, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA.

Mesenchymal stem cells (MSCs) of bone marrow have been proposed for the repair and regeneration of specific tissues such as bone, cartilage, muscle, marrow stroma, tendon, and other connective tissues. However, MSCs are rare in adult bone marrow. It has been difficult to expand MSCs without losing their stem cell properties, thereby impairing the investigation of their behavior and limiting their therapeutic potential. Recently, we have shown that the extracellular matrix (ECM) prepared from marrow stromal cells dramatically enhanced MSC proliferation and effectively retained their stem cell properties (Chen et al, JBMR. 22:1943, 2007). The mechanism of how the ECM positively affects MSC proliferation is unclear. Interestingly, it was observed that cells cultured on the ECM were evenly distributed, whereas cells maintained on plastic grouped into nodules. This finding implies that the ECM may facilitate MSC motion. To confirm this, we applied time-lapse microscopic imaging to track cell migration on the ECM versus that on an uncoated coverslip. In this experiment, human MSCs were first sorted by FACS using an antibody against SSEA-4 a surface marker for MSCs (Gang et al, Blood. 109:1743, 2007). The MSCs were then seeded onto coverslips either uncoated or coated with the ECM made by human marrow stromal cells, at 1400 cells/cm^2, allowing 20-30 cells per viewing field. The motility of cells maintained on an uncoated coverslip or on the ECM was quantified by measuring the velocity, displacement, and frequency of cell-cell contact of migrating cells using a time-lapse individual cell migration assay. Time-lapse images revealed that MSCs cultured on the ECM moved along the direction of collagen fibers, whereas cells cultured on the uncoated coverslip moved randomly. The average velocity of cells on the ECM was 25.6±11.3 μm/hr, which was significantly ($p < 0.001$) higher than the 17.3±4.9 μm/hr on the uncoated coverslip. Interestingly, the average of uninterrupted cell displacement over 24 hrs was 123 μm on the ECM, while 62 μm on the uncoated coverslip. Moreover, time-lapse videos showed that cell-cell collisions occurred less frequently (6 in a 24-hr period) when cells were cultured on the ECM than cells cultured on the uncoated coverslip (27 in a 24-hr period). After 72 hrs of culture, the number of cells expanded on the ECM was at least 3-fold greater than that expanded on the uncoated coverslip. We conclude that the ECM promotes MSC motility, and directs the MSC migration, thereby preventing stem cell cell-to-cell contact inhibition, which in turn results in an increased number of stem cells.

***Disclosures:* *X. Chen**, None.*

This study received funding from: NIH R21.

SA135

See Friday Plenary number F135.

SA136

The Early Osteoctye Marker, E11/gp38 Is Highly Elevated in New Bone Formed in Response to Distraction as Compared to Existing Bone. D. Pacicca[1], A. Zaidi*[2], A. Shah*[3], L. Bonewald[4]. [1]Orthopaedic Surgery, Children's Mercy Hospital, Kansas City, MO, USA, [2]UMKC School of Medicine, Kansas City, MO, USA, [3]Orthopaedic Surgery, UMKC School of Medicine, Kansas City, MO, USA, [4]Oral Biology, University of Missouri-Kansas City, Kansas City, MO, USA.

E11/gp38 is a 40kd membrane protein expressed by cells with dendritic processes. Its expression is highly elevated in embedding osteocytes and E11 expression is also increased in response to mechanical loading. We hypothesized that E11 expression would be elevated in distraction osteogenesis due to enhanced bone formation under tension.

C57Bl/6 mice (n=20) underwent application of a distraction fixator to the right femur with midshaft osteotomy. After latency, femurs were distracted at 0.15 mm per day for 14 days. Radiographs were obtained at days 7, 14, 21 and 28. Animals were sacrificed at either 7, 14, 21 or 35 days postop and femurs were harvested. Serial frozen sections were immunostained for E11 and new bone formation in each specimen was quantified. The average number of cells expressing E11 was counted using 6-8 high power fields per section. Quantification was done for both new bone formed in the distraction site as well as the primary spongiosa. Fluorescent microscopy showed numerous E11 positive osteocytes with abundant dendritic processes in the newly formed distracted bone. In contrast, in normal trabecular bone, there were much fewer E11 positive osteocytes, and these were limited to embedding or recently embedded cells near the surface of the bone, with fewer visible dendrites. The average number of E11 positive cells per unit bone area was significantly elevated in distracted bone, compared to primary spongiosa (138±36 for distracted bone, 56±24 for primary spongiosa, $p=0.0001$).

These data suggest that E11 is a marker for new bone formation and for newly formed or forming osteocytes. Previously it was shown that E11 expression increases in response to mechanical stimulation *in vitro and vivo* (Zhang et al, 2006). Therefore, the elevated expression of E11 during distraction may be not only a reflection of the high number of embedding or recently embedded osteocytes but may also be modulated by the mechanical strains that occur within the distraction site. Further studies that compare fracture healing to distraction in mice with targeted deletion of E11 should help to determine the role of mechanical loading and E11 in new bone formation.

***Disclosures:* *D. Pacicca**, None.*

SA137

TiO_2-scaffolds for Use in Bone Tissue Engineering. R. Sabetrasekh*, G. Fostad*, B. Hafell*, A. Førde*, J. Reseland, S. Lyngstadaas*, H. Haugen*. University of Oslo, Faculty of Dentistry, Department of Biomaterials, Oslo, Norway.

There is an increasing demand for bone repair due to bone lost cause by tumors, infections and trauma. Traditionally, autologous and allogenic bone grafts have been utilized for bone reconstructions. However, along the past decade a paradigm shift is taking place from using tissue graft to biomaterials. In this study we have chosen titanium oxide (TiO_2) as scaffold's material since it has proven to fulfil many of the demands for a scaffold material. The main goal was to improve the mechanical properties of TiO_2 scaffolds, in terms of making stronger, bigger-load bearing devices with the desired three-dimensional geometry for bone tissue engineering.

The polymer sponge method was chosen as method making TiO_2 scaffolds. Fully reticulated polyester based polyurethane foams with 60 ppi were coated with solution of H_2O and TiO_2 powder. The TiO_2 scaffolds were evaluated for the mechanical strength by compression test and the pore morphology was evaluated by SEM and microCT image analysis. Statistic variations were analysed by the standard deviation of four randomised scaffolds from 16 different batches. Different data groups were compared through a two-tailed ANOVA test, where the significant level was set at 0.05. Further more the TiO_2 scaffolds were evaluate the for bone tissue engineering by growing the murine preosteoblasts (MC-3T3-E1) on the TiO_2 scaffolds. Cytotoxic effect of the TiO_2 scaffolds on the osteoblasts was determined by lactate dehydrogenase activity in the culture medium and cell morphology was evaluated by SEM.

The scaffolds had an average pore size of 582.07μm and a porosity of 94.3% which gives us the opportunity to further coating and improvement of strength, still maintaining optimal conditions for osseoconductivity and vascular growth. The BET Surface Area was found to be 5.0664 m²/g. This gives a greater attachment surface for cells compared to the other commercial scaffolds. The TiO_2 scaffolds had compressive strength in the range of 0.6MPa to 1.2MPa after. These scaffolds had all porosity higher than 90%. The interconnectivity was also found to be high. We observed less than 5% cytotoxic effect of the TiO_2 scaffolds on the osteoblasts.

Study has showed that it is possible to produce TiO_2 scaffolds which are both mechanical loadable, have high reproducibility and low cytotoxicity. TiO_2 scaffolds with porosity of 90 % had compressive strength higher than 1 MPa. Since the porosity is far above what is necessary for osseointegration, reduction of porosity to reinforce the scaffolds even further is possible.

***Disclosures:* *R. Sabetrasekh**, None.*

This study received funding from: University of Oslo, Norway.

SA138

Bone Marrow Stromal Cells and Osterix Contributing to Osseointegration of Dental Implants. X. Beiyun*[1], J. Zhang*[1], E. Brewer*[1], Q. Tu[1], M. Wieland*[2], J. Chen[1]. [1]General Dentistry, Tufts University, School of Dental Medicine, Boston, MA, USA, [2]Insititue Straumann AG, Basel, Switzerland.

The purpose of this study is to identify the source and potential of cells involved in the processes of osteogenesis after implantation, and to elucidate the roles of osterix (Osx) in osteogenic differention at the interface between host bone and titanium dental implants.
BSP9.0Luc/ACTB-EGFP mice contain a luciferase reporter gene driven by BSP promoter and an enhanced green fluorescent protein (EGFP) driven by a beta-actin promoter. Bone marrow stromal cells (BMSCs) isolated from these transgenic mice were transplanted into 4-week-old nude mice through intracardiac injection. Five weeks after transplantation, titanium implants (1.05 mm in diameter) were inserted into the femurs of these nude mice. The femurs were dissected at d7 (day7) and d21 after implantation. H&E staining and immunohistochemical (IHC) staining were performed. BSP/TVA mice, in which an avian retroviral receptor gene is hooked to a mouse BSP promoter, were divided into two groups. Titanium implants were inserted into bilateral femurs after local administration of RCAS/OSX virus or empty virus as control, respectively. The femurs were dissected for microCT, RT-PCR, H&E staining and IHC staining at d7 and d14 after implantation.
In BMSCs transplanted nude mice, IHC staining showed that luciferase, BSP and GFP could be detected in the newly formed bone at d7 and d21. The ratio of luciferase positive cells to GFP positive cells detected at d7 was higher than that at d21. In BSP/TVA mice, 14 days after implantation, microCT analysis showed an increased bone mineral density at the bone-to-implant interface in RCAS/Osx group compared with the control group. As shown by semiquantitative RT-PCR analysis, levels of Osx, BSP and osteocalcin (OC) were higher in RCAS/Osx group than in the control group at d7 and d14. In both groups, levels of BSP and OC were higher at d14 than at d7 after implantation. Histomorphometrical analysis demonstrated increased percentage of bone-implant contact in RCAS/Osx group compared with control group at d7 and d14. Immunohistochemical staining revealed active expression of BSP and OC in the newly formed bone in RCAS/Osx group at d7 and d14.
In conclusion, exogenous BMSCs underwent osteogenic differentiation and participated in the osseointegration after implantation. Overexpression of Osx could accelerate bone regeneration process at bone-to-implant interfaces.

Disclosures: ***X. Beiyun****, Institute Straumann AG, Basel, Switzerland 3.*
This study received funding from: Institute Straumann, AG, Basel, Switzerland.

SA139

Regulation of Intracellular Signaling of MC3T3-E1 Osteoblasts by the Extracellular Matrix. P. A. Jones*, R. L. Duncan. Department of Biological Sciences, University of Delaware, Newark, DE, USA.

Osteoblast adhesion to the extracellular bone matrix (ECM) is critical to the response of these cells to hormonal and mechanical stimuli. However, the signaling mechanisms activated by attachment and growth of osteoblasts on different ECM's are poorly understood. We postulate that direct integrin attachment to different ECM proteins will result in altered calcium signaling and cell function. Early signaling responses mediated by attachment of type I collagen and fibronectin to MC3T3-E1 osteoblastic cells were investigated using atomic force microscopy (AFM) and calcium imaging. Using AFM, we determined that the average unbinding force of fibronectin to the cell was 148.3±3.3 pN, while the average unbinding force of collagen to the cell was 97.8±4.4 pN. When the fibronectin coated tip was allowed to stay in contact with the cell for 60 seconds before measuring the force of unbinding, the measured unbinding force increased two-fold. Using calcium imaging techniques, the global intracellular calcium ($[Ca^{2+}]_i$) response of MC3T3-E1 cells elicited by soluble ECM proteins was determined. Addition of soluble fibronectin to MC3T3-E1 cells produced a slow, sustained increase in $[Ca^{2+}]_i$, whereas type I collagen and the ECM associated peptide Arg-Gly-Asp-Ser (RGDS) caused a smaller, more rapid increase in intracellular calcium. The RGDS motif is contained in the sequence of fibronectin and the failure of this motif to elicit the same response as the full length fibronectin molecule indicates that other cell binding regions of fibronectin are essential to this $[Ca^{2+}]_i$ response. The $[Ca^{2+}]_i$ response to soluble ECM proteins was dependent on both the entry of calcium from the extracellular environment as well as the release of calcium from intracellular stores. The binding of osteoblasts to different ECM substrates also affects growth, as MC3T3-E1 cells grown on fibronectin exhibited increased proliferation as compared to the same cells grown on type I collagen. Further, this increase in proliferation on fibronectin appeared to be mediated by the L-type voltage sensitive calcium channel (L-VSCC), since the L-VSCC inhibitor, nifedipine, reduced proliferation on fibronectin coated substrates. Addition of nifedipine to osteoblasts grown on type I collagen did not have a significant effect on proliferation. Consequently, the attachment of osteoblasts to fibronectin appears to cause an increase in cell proliferation that is calcium dependent and mediated through the L-VSCC. Elucidating the signaling mechanisms in osteoblasts that are mediated by the surrounding extracellular matrix will provide important insight into the mechanisms of bone formation and maintenance.

Disclosures: ***P.A. Jones****, None.*
This study received funding from: National Institutes of Health.

SA140

DMP1 Fragments Interact Differently with Hydroxyapatite: Insights into Mechanism of Action. A. Boskey[1], C. Qin*[2], Y. Sun*[2], W. T. Butler*[2], H. Taleb*[1], D. Redfern*[3], A. Gericke*[3]. [1]Musculoskeletal Integrity Program, Hospital for Special Surgery, New York, NY, USA, [2]Department of Biomedical Sciences, Baylor College of Dentistry, Texas A & M University System Health Science Center, Dallas, TX, USA, [3]Department of Chemistry, Kent State University, Kent, OH, USA.

Dentin matrix protein 1 (DMP1) is an acidic noncollagenous protein critical for hydroxyapatite (HA) mineral deposition in bones and teeth. The purpose of this study was to elucidate the mechanism of action of this process. DMP1 is present in mineralized tissues as 3 fragments: the NH2-terminal (37 kDa) and COOH-terminal (57 kDa) portions and a proteoglycan fragment, DMP1-PG that is a chondroitin-sulfated 37-kDa DMP1 fragment. The effects of each of these fragments on HA formation were examined in a cell-free solution study, and the interaction of the fragments with HA determined by attenuated reflection infrared spectroscopy (ATR-FTIR). The ATR-FTIR experiment involved interaction of the peptide solution with HA or Ca^{+2}, followed by the characterization of the resulting secondary structure changes based upon the analysis of the protein amide I band envelope. In solution, at 10, 12.5 and 25 ug/ml both the 37kDa and 57kDA fragments acted as HA formation nucleators; in contrast, DMP1-PG was an effective inhibitor at 10 ug/ml, but at higher concentrations had a lesser effect. The intact protein (full length DMP1) expressed in bovine marrow stromal cells was also an inhibitor. Conformational studies showed that the full length DMP1 in the presence of HA or Ca^{2+} has a slightly increased β-sheet secondary structure content relative to the protein in the absence of HA or Ca^{2+}. Dynamic Light Scattering experiments showed DMP1 aggregates in the presence of Ca^{2+}. The 37 kDa fragment showed even greater aggregation. The 57 kDa fragment had increased α-helical secondary structure in the presence of HA and Ca^{2+}. In conclusion, these results suggest that in mineralizing tissues DMP1 fragments can have distinct regulatory effects on mineralization depending on their composition, via an interaction with HA. Thus, in the DMP1 KO where there is a decrease in mineral content, the absence of the nucleating fragments, appear to be important, while the DMP1-PG fragment may be more important for cell-matrix interactions.

Disclosures: ***A. Boskey****, None.*
This study received funding from: NIH DE04141.

SA141

See Friday Plenary number F141.

SA142

Bone Sialoprotein (BSP) Deficiency Impairs Osteoclast Differentiation and Resorption *in vitro*. M. Boudiffa*[1], N. Wade-Gueye*[1], A. Guignandon*[1], M. Linossier*[1], A. Vanden-bossche*[1], A. Anginot[2], M. Lafage-Proust[1], J. Aubin[3], P. Jurdic[2], L. Vico[1], L. Malaval[1]. [1]LBTO, INSERM 890, Saint-Etienne, France, [2]Umr cnrs 5161, inra 1237, ENS, Lyon, France, [3]Dept of Molecular Genetics, Faculty of Medicine, University of Toronto, Toronto, ON, Canada.

Like Osteopontin (OPN), Bone sialoprotein (BSP) belongs to the SIBLING (Small Integrin Binding Ligand N linked Glycoproteins) family, whose members interact with bone cells and mineral. We have previously shown that BSP knockout (KO) mice have a higher bone mass with low bone formation and reduced osteoclast (oc) surface as compared to wild type (WT) mice. Because this phenotype suggests a defect in oc recruitment and function, we studied the differentiation and activity of KO oc *in vitro*.
Oc precursor-containing murine spleen cell cultures were grown in the presence of RANKL and M-CSF and analysed at day (d)2 (appearance of TRACP+ cells), d5 (onset of multinucleation) or d7 (peak number- nb- of multinucleated oc). Fixed cells and extracted RNAs were used for Tartrate Resistant Acid Phosphatase (TRACP) or immunostainings (IS) and RT-PCR, respectively. IS confirmed the strong BSP expression in WT oc and its absence in mutants. At d7, TRACP+ cell nb (1150 ± 104 vs 1486 ± 93 n=12, $p<0.02$) and multinucleated oc nb (≥3 nuclei) (66 ± 11 vs 447 ± 35 n=12, $p<0.0001$) were lower in KO than in WT. Addition of 40 nM exogenous fibronectin, 80nM BSP or >400nM recombinant OPN throughout the culture time restored multinucleated oc nb in KO to WT values. However, none of these supplements restored TRACP+ cell nb at d2 to the WT level. Thus, BSP seems to be required for oc differentiation at two levels : intracellular BSP is needed for the commitment of precursors to the oc lineage and extracellular BSP is required for multinucleation. Further, image analysis of actin-stained prefusion oc (d5) suggested a higher podosome turnover in KO with more but smaller podosomes (85 ± 51 vs 145 ± 18, n=30, $p<0.05$) than in WT. Oc gene expression was markedly altered in KO compared to WT cells, with expression reduced for cell adhesion genes (beta3 integrin & OPN) but increased for promigratory genes (MMP2 & 9), TRACP and cathepsin K . In vitro resorption assays were performed on either BSP-containing dentin slices or BSP-free mineral coated slides (Osteologic™) in presence of RANKL and MCSF for 2 d. No difference in pit number (23 ± 4 vs 24 ± 9, n=6, p= 0.79) or pit area was seen between WT and KO on dentin slices. However, pit formation by KO oc was markedly reduced on BSP-free discs (9±1 vs 22 ± 3 , n=15, $p<0.0001$). In conclusion, BSP appears essential for oc formation and resorption *in vitro*, confirming our in vivo findings. Moreover, the down-regulation of OPN expression in KO oc suggests a physiological interplay between these two SIBLING family members which remains to be clarified.

Disclosures: ***M. Boudiffa****, None.*
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SA143

Sequestration, Proliferation and Differentiation of Osteoblasts in Hydrogels for Tissue Engineering Applications. M. Dadsetan*[1], T. E. Hefferan[1], A. Heine-Geldern*[1], M. Benedikt*[1], D. Gaustad*[1], J. Herrick*[1], T. C. Spelsberg[2], L. Lu*[1], A. Maran[1], M. J. Yaszemski[1]. [1]Orthopedic Surgery, Mayo Clinic, Rochester, MN, USA, [2]Biochemistry and Molecular Biology, Mayo Clinic, Rochester, MN, USA.

Bone is an organ that can regenerate under normal physiological conditions; however, after trauma or surgical resection for removal of bone tumors the remaining void impedes bone regeneration. In order for bone regeneration to proceed, osteoblasts must be present and functional; therefore, under these abnormal physiological conditions implementation of tissue engineering strategies to promote bone regeneration would be beneficial to provide the necessary functional cells. The osteoblasts must arrive at the site, be viable, differentiate, and eventually form bone. Therefore, our objective was to test if osteoblasts would remain viable in a hydrogel and if these cells would be able to proliferate and differentiate.

For these studies we utilized a photocrosslinkable hydrogel, oligo-(polyethylene glycol), (OPF), to encapsulate normal human osteoblasts (hFOB). The cells were cultured under static or dynamic conditions in a rotating wall vessel bioreactor (Synthecon/ RCCS-4DQ) containing DMEM/F12, 10% FBS, 1% penicillin/streptomycin and 0.6% geneticin for 3, 7 or 14 days. Rotation was set at 0 rpm for static cultures and 25 rpm for dynamic cultures. Viability of the cells was determined by uptake of calcein from a Live/Dead Kit (Molecular Probes) and proliferation was quantified by measuring DNA content using the PicoGreen Kit (Molecular Probes). Alkaline phosphatase activity was utilized as the marker of osteoblast differentiation.

The hFOB cells demonstrated calcein uptake after encapsulation in the hydrogel and during the 14 day time course, thus indicating viability throughout the experimental period. There was a significant effect of time ($p<0.05$) on the proliferation of the osteoblasts with the greatest proliferation occurring at day 3. Interestingly, there was no significant effect of culture conditions with the cells proliferating in both the static and dynamic cultures. However, there was a significant increase ($p<0.001$) in alkaline phosphatase activity for the osteoblasts grown in dynamic culture with 1.6 fold increase between days 3 and 7, and a 1.4 fold increase between days 3 and 14.

These studies have shown that osteoblasts can be successfully encapsulated in an OPF hydrogel and maintain three functions which are crucial for tissue regeneration: viability, proliferation and differentiation. Thus, OPF hydrogels can be considered as a candidate material to sequester osteoblasts and deliver these cells to sites where bone regeneration is needed.

Disclosures: ***T.E. Hefferan****, None.*
This study received funding from: NIH.

SA144

Identification of Dentin Sialoprotein-Phosphophoryn Cleavage Site. H. H. Ritchie*. Cariology, Restorative Sciences and Endodontics, University of Michigan School of Dentistry, Ann Arbor, MI, USA.

Introduction: Dentin sialoprotein (DSP) and phosphophoryn (PP) are the two noncollagenous proteins classically linked to dentin mineralization, but more recently found in bone, kidney and salivary glands. Point mutations in DSP-PP gene are linked to Dentinogenesis imperfecta. These two proteins are derived from a single copy DSP-PP gene. Because native PP has a DDPN N-terminal sequence, we hypothesized that $SMQG^{447}|D^{448}DPN$ is the specific cleavage site for generating DSP_{430} and PP_{240}. To test this hypothesis, our Objective was to generate DSP-PP_{240} containing mutated G^{447} and follow the expression and processing of this mutated precursor protein. Methods: To achieve this goal, we used site-mutagenesis to generate G^{447} mutations in DSP-PP_{240} cDNA in pGEM7Z(+), which were subsequently subcloned into pVL1392 baculovirus vector for protein expression. Results: Wild type and mutated DSP-PP_{240} precursor proteins are secreted into the extracellular space in the baculovirus expression system. Using MS, MS/MS analysis on the recombinant PP sample, the N-terminal sequence was determined to be DDPN. DSP-PP_{240} cDNA containing G^{447} mutant yielded a DSP-PP_{240} precursor protein, which was not further processed into DSP_{430} and PP_{240}. Conclusion: $G^{447}|D^{448}$ is the cleavage site for generating DSP_{430} and PP_{240} from the wild type precursor molecule. This work was supported by NIH DE11442-9 to HHR.

Disclosures: ***H.H. Ritchie****, None.*
This study received funding from: NIDCR.

SA145

See Friday Plenary number F145.

SA146

Do Type I Collagen Homotrimers Contribute to Osteoporosis by Altering Bone Remodeling? S. Han*[1], E. Makareeva*[1], N. V. Kuznetsova*[1], A. M. DeRidder*[1], M. B. Sutter*[1], D. J. McBride*[2], C. L. Phillips*[3], U. Schwartze*[4], J. M. Pace*[4], P. H. Byers*[4], R. Visse*[5], H. Nagase*[5], S. Leikin[1]. [1]Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD, USA, [2]School of Medicine, University of Maryland, Baltimore, MD, USA, [3]Department of Biochemistry, University of Missouri, Columbia, MO, USA, [4]Department of Pathology, University of Washington, Seattle, WA, USA, [5]Kennedy Institute of Rheumatology, Imperial College London, London, United Kingdom.

Normal type I collagen molecules are heterotrimers of two α1(I) and one α2(I) chains, but insufficient or abnormal synthesis of the α2(I) chain can result in α1(I) homotrimers, e.g., in fetal tissues and various pathological conditions. In particular, synthesis of a small fraction of α1(I) homotrimers in individuals with a polymorphism in the SP1 binding region of COLIA1 is discussed in the literature as a factor contributing to fracture in age-related osteoporosis. It is not clear, however, how to reconcile such an effect with outcomes of complete heterotrimer replacement by α1(I) homotrimers. Severe Osteogenesis Imperfecta was reported in a patient with synthesis of nonfunctional α2(I) chains, but no dramatic bone pathology was observed in patients completely lacking the α2(I) chain synthesis. If normal bone formation is possible with the complete replacement of the heterotrimers, how can just a small fraction of the homotrimers contribute to age-related bone fragility? To address this question, we investigated murine and human α1() homotrimers and their mixtures with the corresponding heterotrimers. The most significant findings with potential implications to tissue pathology were: (a) the segregation of the homo- and heterotrimers at a subfibrillar level and (b) the significantly reduced susceptibility of the homotrimers to cleavage by tissue collagenases. A more detailed study with rhMMP-1 revealed that the lack of the α2(I) chain does not alter the enzyme binding but hinders opening of the triple helix necessary for presenting the unwound chains to the catalytic site. In mixtures, rhMMP-1 degraded the normal type I heterotrimers before noticeable cleavage of the homotrimers. We hypothesize that an initial deposition of α1(I) homotrimers may not reduce the bone strength. However, their subfibrillar segregation and selective proteolytic degradation of the heterotrimers may result in accumulation of defects after multiple tissue remodeling cycles, altering the bone matrix quality.

Disclosures: ***S. Leikin****, None.*
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SA147

Various Roles of Syndecan Family in Osteoblastic Cells. T. Ikeo[1], A. Kamada[1], Y. Yoshikawa[1], E. Domae*[1], S. Goda*[1], I. Tamura*[1], A. Kawamoto*[1], J. Okazaki*[1], Y. Komasa*[1], Y. Takaishi[1], T. Miki[2], T. Fujita[1]. [1]Osaka Dental University, Osaka, Japan, [2]Osaka City University, Osaka, Japan.

Syndecans are transmembrane heparan sulfate proteoglycans known to bind both other extracellular matrix molecules and certain growth factors, thus participating in a number of fundamental biological processes, including cell adhesion and migration, and the binding and activation of growth factors, enzymes, and inhibitors. The syndecan family in vertebrates comprises four members, syndecan-1, syndecan-2 (fibroglycan), syndecan-3 (N-syndecan), and syndecan-4 (ryudocan). Previously, we showed both protein and gene expression of syndecan-1, -2 and -4 in normal human osteoblasts. To understand the role of syndecans in osteoblasts, the effect of a bone- resorbing cytokine, IL-1beta, and differentiation-stimulated reagent, beta-glycerophosphate (BGP), on syndecan expression were examined using human osteoblast-like cell SaOS2.

Using western blot analysis, it was found that IL-1b caused a marked appearance of a certain heparan sulfate proteoglycan corresponding to syndecan-4. Furthermore, the mRNA expression levels of syndecan family were measured using real-time quantitative RT-PCR technique, and demonstrated the gene expression of syndecan-1, -2 and -4 in the human osteoblast-like cells.

When cells were incubated with IL-1beta for 24 h, there were significant decrease in mRNA levels of syndecan-1 and syndecan-2 compared with time match controls, though the expression of syndecan-4 was markedly increased. These results demonstrate that syndecan-1, -2 and -4 are synthesized in human osteoblastic cells, and syndecan-4 gene transcription is rapidly responsive to an inflammatory mediator. On the other hand, addition of BGP increased syndecan-1 and -2 expression but not syndecan-4.Hence, it is indicated that syndecan-1 and -2 may be involved with the differentiation and maturation of osteoblasts, while syndecan-4 may be important in the intracellular signal transduction.

Disclosures: ***T. Ikeo****, None.*

SA148

Immunohistochemical Localisation of Sclerostin in Human Trabecular Bone from Fragility Hip Fracture Patients. A. Papadopoulos*, L. Truong*, J. S. Kuliwaba*, N. L. Fazzalari. Bone and Joint Research Laboratory, Institute of Medical and Veterinary Science, Adelaide, South Australia, Australia.

Osteocytes, the most abundant cell type in bone, are ideally located to influence bone remodelling through their syncytial relationship with surface bone cells. The expression of osteocyte-derived factors such as sclerostin, an inhibitor of bone formation and subsequent bone mineralisation, in osteocytes imply that these bone cells may regulate mineralisation, and hence, bone material properties for bone strength.
This study analysed the immuno-localisation of sclerostin in human femoral trabecular bone obtained from postmenopausal females undergoing total hip arthroplasty following a fractured neck of femur (#NOF; n = 10, mean age 78.4 ± 5.7 years) and a non-fracture control (NFC) group, primary hip osteoarthritis (n = 8, mean age 78.9 ± 6.1 years). Using the streptavidin biotin peroxidase method of immunohistochemistry in paraffin-embedded bone tissue, positive nuclear and cytoplasmic sclerostin immunostaining were observed specifically in the osteocytes, as well as in the canaliculi and lacunar walls, as reported in published literature. The #NOF group had significantly less bone volume fraction (BV/TV; #NOF = 7.4 ± 4.1 %, NFC = 11.5 ± 3.2 %, $p < 0.04$). No significant differences were found in the number of positive-stained and negative-stained osteocytes per bone area (N.POS.Ot/BV and N.NEG.Ot/BV, respectively), number of empty lacunae per bone area (N.Em.Lc/BV), and in the total number of lacunae per bone area (Total Lc.N/BV) observed, compared to NFC. The percentage of positive (N.POS.Ot/Total Lc.N) and negative (N.NEG.Ot/Total Lc.N) osteocytes, and percent of empty lacunae (N.Em.Lc/Total Lc.N) were similar between the two groups. Interestingly, percent positive-stained osteocytes differed significantly to negative-stained osteocytes in the #NOF group (28.0%POS.Ot vs. 69.3%NEG.Ot, $p < 0.009$), in contrast to similar percent positive-negative in the NFC group (47.7%POS.Ot vs. 46.3%NEG.Ot).
The interesting finding of decreased percent positive osteocytes in the #NOF group, compared to similar percent positive-negative osteocytes in the NFC group, may suggest bone in #NOF individuals is undermineralised, as sclerostin protein expression has been found to be expressed only in mature osteocytes in fully-mineralised bone tissue (Poole *et al*, 2005). These results may further inform understanding the mechanism of decreased mineralisation of bone in fragility fracture patients (Sutton-Smith *et al*, 2008). Studies of the role of osteocytes and their derived factors in normal and bone pathology will aid in furthering the understanding of the pathogenesis of fragility fractures and the efficacy of new pharmaceuticals to treat osteoporosis.

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SA149

See Friday Plenary number F149.

SA150

Proteins Involved in Mineralization Expressions Perturbations in Bones and Teeth of Msx2 Mutant Mouse Mandibles. A. Bolanos*[1], D. Hotton*[1], A. Agalliu*[1], M. Molla*[1], B. Castaneda*[1], B. Robert*[2], F. Lézot*[1], A. Berdal*[1]. [1]Laboratoire de Physiologie Orale Moléculaire (Eq. 5), INSERM UMRS 872, Centre de Recherche des Cordeliers, Paris, France, [2]Pasteur Institute - URA 2578 CNRS, Paris, France.

Homeobox genes of Msx family are implicated in early craniofacial skeleton development. The part of these genes later in the biomineralization has been underestimated while their expressions were described in all mineralized tissues forming cells. In contrast to Msx1 null mutant (KO) mouse that died at birth, the Msx2 KO mouse survives allowing the analysis of the impact of Msx2 absence on the expression of proteins implicated in the biomineralization.
In the present study, using immuno-histochemistry, in situ hybridization and quantitative RT-PCR, expressions of calbindin-D28K, osteocalcin, osteopontin, bone sialoprotein, collagen type I, amelogenin and ameloblastin were analyzed in mandible bones and teeth comparatively between Msx2 KO, Msx2 heterozygous and wild type mice.
Results show that all protein expressions are perturbed in the Msx2 KO mouse either in intensity as osteocalcin, collagen type I and amelogenin or in pattern as calbindin-D28K. In the Msx2 heterozygous mouse, the expression levels of some proteins implicated in the mineralization are also perturbed but evidence an opposite modulation compared to Msx2 KO. Indeed, amelogenin expression is increased in the dental epithelium of Msx2 heterozygous similarly to osteocalcin, bone sialoprotein and osteopontin in bones of the mandible. In Msx2 heterozygous mouse, the most striking result is the perturbation of calbindin-D28K expression pattern with an important level observed in the dental pulp.
To conclude, this study evidences that Msx2 genetic status (KO, heterozygous and wild type) has important repercussions on the expression (level and pattern) of proteins implicated in the mineralization, so on the mineralization rank itself.

***Disclosures:* *A. Bolanos**, None.*
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SA151

Circulating Fibronectin Affects Bone Matrix. A. Bentmann*[1], N. Kawelke*[1], D. Moss*[2], I. Nakchbandi[1]. [1]Max-Planck Institute for Biochemistry and University of Heidelberg, Heidelberg, Germany, [2]Research Center Karlsruhe (ANKA), Karlsruhe, Germany.

Targeted deletion of fibronectin production by differentiating osteoblasts affects their function, but does not result in a significant decrease in the amount of fibronectin in the bone matrix or in a change in bone matrix. The liver is a major source of fibronectin. It also produces a number of proteins that are able to infiltrate the bone such as albumin and alpha2-HS-glycoprotein. We therefore examined the role of circulating fibronectin originating from the liver in bone.
Plasma fibronectin was labeled with Oyster-500 and injected in mice intraperitoneally. Examination of bone sections revealed infiltration of labeled fibronectin throughout the bone. This was not limited to areas of active bone mineralization, determined by injecting alizarin complexone prior to injecting labeled fibronectin. We then performed matings of fibronectin floxed mice with mice carrying the Cre gene under the control of the Mx or the albumin promoters in order to delete fibronectin production by the hepatocytes. Both lines showed a decrease in circulating fibronectin by more than 95% (Circulating fibronectin in the Mx line was 4.6±0.1 vs. 6.2±0.4 mg/L in the albumin line, $p<0.01$). Fibronectin staining in the liver-specific fibronectin knockouts revealed a significant decrease in the amount of fibronectin in the bone matrix that was confirmed by Western blotting.
The deletion of fibronectin in the liver was also associated with 13% decrease in bone mineral density in the Mx-cre harboring line (conditional knockouts: 177.9±5.4 vs. controls: 205.4±6.2 mg/cm^3, n=24 and 34, $p<0.005$). There were no clear changes on either dynamic or static bone histomorphometry, however. Infrared microspectroscopy was therefore performed to determine whether structural changes could be seen in the matrix itself. The ratio of mineral to matrix was significantly decreased by 12% in the conditional knockout mice compared to littermate controls (Phosphate to protein ratio in conditional knockouts: 8.76±0.20 vs. controls: 9.91±0.32, n=6 mice per group and 9 measurements per mouse, $p<0.005$), suggesting that the loss of fibronectin in the matrix affects its mineral content.
In summary, circulating fibronectin originating from the liver infiltrates the bone matrix, where it affects the bone mineral density and mineral content in the bone. These effects take place in the absence of clear changes in the bone forming or bone resorbing cells. Thus fibronectin originating from the liver is needed for a normal matrix mineralization. This is in contrast to the effect of the deletion of fibronectin in osteoblasts showing that its production by them is required for a normal osteoblastic function without affecting the mineralization of matrix.

***Disclosures:* *A. Bentmann**, None.*

SA152

PGD2 Downregulates MMP-1 and MMP-13 Expression in Human Osteoarthritic Chondrocytes. N. Zayed*, H. Afif*, N. Chabane*, J. Martel-Pelletier*, J. Pelletier*, H. Fahmi*. CHUM, Université de montréal, Montreal, QC, Canada.

Purpose: Prostaglandin (PG) D2, a cyclooxygenase (COX) metabolite, has been shown to play critical roles in multiple physiological processes including inflammation. Matrix metaloproteinases (MMPs), in particular MMP-1 and MMP-13 produced by chondrocytes, are major proteases responsible for cartilage degradation during osteoarthritis. This work was designed to investigate the effects of PGD2 on interleukin-1 (IL-1)-induced MMP-1 and MMP-13 expression in human chondrocytes.
Methods: Cultured chondrocytes were stimulated with IL-1 in the absence or presence of PGD2 and MMP-1 and MMP-13 protein production was evaluated by ELISA. mRNA expression and promoter activity were analyzed by real-time RT-PCR and transient transfection experiments, respectively. The expression of PGD2 receptors, DP1 and CRTH2 was investigated using RT-PCR and Western blotting.
Results: PGD2 dose-dependently decreased IL-1-induced MMP-1 and MMP-13 protein expression. Moreover, PGD2 prevented IL-1-induced MMP-1 and MMP-13 mRNA expression as well as MMP-1 and -13 promoter activation, indicating that this effect occurs at the transcriptional level. We also demonstrated that chondrocytes expressed both PGD2 receptors: DP1 and CRTH2.
Conclusions: These data indicate that PGD2 inhibits IL-1-induced MMP-1 and MMP-13 production by human chondrocytes. Therefore, PGD2 may play an important role in the chondrocyte response to inflammatory stimuli and may protect cartilage integrity.

***Disclosures:* *N. Zayed**, None.*
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SA153

See Friday Plenary number F153.

SA154

Cathepsin K Expression During Enamel Formation. C. E. Tye, Y. Ding*, Y. P. Li, J. D. Bartlett*. Department of Cytokine Biology, Forsyth Institute, Boston, MA, USA.

Dental enamel is unique in that it starts forming as a soft, protein-rich substance and ends as a hard, almost protein-free mineral. It is well established that enamel matrix proteins are degraded during enamel formation and these degraded proteins are removed from the matrix to allow for the increase in mineral content. Proteolytic processing is critical for proper enamel formation as homozygous mutation of either of the resident enamel proteases, matrix metalloproteinase-20 (MMP-20) or kallikrein-4 (KLK-4), causes *Amelogenesis Imperfecta* where the enamel is hypomineralized and protein-rich. Our objective is to determine if in addition to KLK-4 and MMP-20, other proteases may be involved in enamel formation. Cathepsin K has been shown to be important in the degradation of the collagenous matrix during bone resorption and we therefore investigated if cathepsin K may play a role in the degradation of the enamel matrix. RNA from molars of 3-11 day-old mice (secretory to maturation stage) was collected and expression of cathepsin K was assessed and quantified by RT-PCR and qPCR. High levels of cathepsin K expression were observed across amelogenesis with highest expression in mature enamel organ (6.35-fold increase over secretory stage). Immunohistochemical (IHC) staining of wild-type mouse incisors confirmed cathepsin K expression by ameloblasts. Cathepsin K was found to rapidly degrade recombinant amelogenin *in vitro* indicating a possible role for cathepsin K in enamel protein degradation. Cathepsin K may degrade the enamel proteins within the lysosomes once peptides are removed from the matrix, and it is possible that cathepsin K is secreted into the matrix to degrade the proteins during the maturation stage of enamel development. Examination of incisors from cathepsin K-null mice however, showed no significant alteration to prism pattern as assessed by scanning electron microscopy. Microhardness testing of enamel from cathepsin K-null mice revealed that loss of cathepsin K expression did not significantly alter enamel hardness. Our results suggest that cathepsin K is expressed by ameloblasts and may be part of a redundant pathway necessary to degrade proteins that are resorbed from the enamel matrix.

Disclosures: ***C.E. Tye****, None.*

SA155

Identification of Dipeptidyl Peptidase I as a Potential Activator of Kallikrein-4. C. E. Tye[1], C. T. Pham*[2], J. D. Bartlett*[1]. [1]Department of Cytokine Biology, Forsyth Institute, Boston, MA, USA, [2]Division of Rheumatology, Washington University School of Medicine, St. Louis, MO, USA.

Kallikrein-4 (KLK-4) is a serine protease expressed during the maturation stage of enamel development and proteolytic processing of the enamel matrix by KLK-4 has been found to be critical for proper enamel formation. Homozygous mutation of KLK-4 causes *Amelogenesis Imperfecta* where the enamel is hypomineralized and protein-rich. KLK-4 has also been found to be overexpressed in prostate and ovarian cancer. The family of KLKs are synthesized as prepro-enzymes that are proteolytically processed to secreted pro-forms via the removal of their signal peptide. The secreted inactive pro-KLKs are then activated extracellularly by specific release of their amino-terminal propeptide. Activation of the pro-forms of the family of KLKs has been well studied; however, identification of the activator of KLK-4 remains elusive. The tooth specific protease matrix metalloproteinase-20 (MMP-20) has been shown to activate KLK-4 *in vitro*; however, KLK-4 and MMP-20 expression overlap only briefly during enamel development suggesting another protease activates KLK-4 *in vivo*. Dipeptidyl peptidase I (DPP I) is an ubiquitously expressed aminopeptidase that sequentially removes two N-terminal amino acid residues from folded proteins and was demonstrated to activate proenzymes of several chymotrypsin-like serine proteases. In this study we sought to examine DPPI expression in mouse enamel organ and determine if DPPI could act as an activator of KLK-4. RNA from molars of 3-11 day-old mice (secretory to maturation stage) was collected and expression of DPPI was assessed and quantified by RT-PCR and qPCR. High levels of DPPI expression was observed across amelogenesis with highest expression in mature enamel organ (11.4-fold increase over secretory stage). Immunohistochemical (IHC) staining of wild-type mouse incisors confirmed DPPI expression by ameloblasts. Using a fluorogenic substrate specific for KLK-4, DPPI was found to activate pro-KLK-4 *in vitro*. Examination of incisors from DPPI-null mice by scanning electron microscopy showed an altered prism pattern and microhardness testing of enamel from DPPI-null mice revealed that loss of DPPI expression caused a decrease in enamel hardness. Our results demonstrate that DPPI is highly expressed by maturation stage ameloblasts and may be involved in the activation of KLK-4. Identification of the *in vivo* activator of KLK-4 is important not only for furthering the understanding of enamel formation but also has exciting potential as a therapeutic target for both ovarian and prostate cancer.

Disclosures: ***C.E. Tye****, None.*

SA156

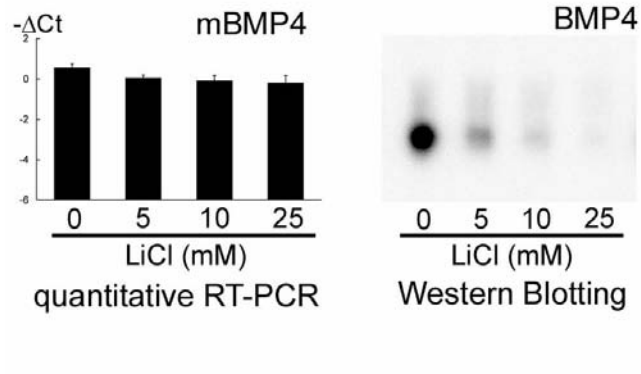
Canonical Wnt Signaling Suppresses BMP4 Accumulation from C3H10T1/2 Cells Transfected with BMP4 Expression Vector. K. N. Kishimoto, E. Itoi*. Department of Orthopaedic Surgery, Tohoku University School of Medicine, Sendai, Japan.

Lithium chloride is known as an inhibitor of glycogen synthase kinase-3 beta (GSK-3beta). Inhibition of GSK-3beta leads to accumulation of beta-catenin and activation of canonical Wnt signaling. The purpose of this study is to analyze the effect of canonical wnt signal on the expression of BMP4 and other related factors during chondrogenic differentiation of C3H10T1/2 induced by BMP4 gene transfer.

C3H10T1/2 cells transfected with mouse BMP4 expressing plasmid was used for experiments. Plasmid was transfered by electroporation in the 4 mm cuvette at 500 V, 2 ms and 2 pulses using T-820 electroporator (BTX). A micromass formed with a drop of 10^5 transfected cells was allowed to adhere for 90 min and filled with 1 ml of medium containing 1% FBS, BSA, ascorbic acid 2 and ITS+. LiCl were added at 5,10 and 25 mM. The effect of LiCl was confirmed by luciferase activity of wnt signaling reporter construct TOPflash. For quantitative RT-PCR, total RNA was isolated from each micromass. Real-time quantitative analyses were carried out with SYBR green I dye and ABI 7700 sequence detector. The culture medium was subjected to Western-blot analysis using anti-BMP4 antibody (SantaCruz) which react with both pro-BMP4 and BMP4. Micromass on 12-well plate were fixed with cold ethanol and immuno-staining was carried out with the same anti-BMP4 antibody and anti-mouse rabbit antibody conjugated with Alexa fluor 555 (Invitrogen).

LiCl treatment increased the luciferase activity of TOP flash reporter assay in a does-dependent manner. The expression of BMP4 mRNA was not affected by LiCl. However, marked decrease of BMP4 protein by the addition of LiCl were seen in western blotting.(Figure) Also, immuno-staining for BMP4 on micromass showed decreased accumulation of BMP4 protein by LiCl. Under the presence of LiCl, alcian blue stainings of micromass were inhibited in a does-dependent manner. Aggrecan, col2a1 and BMP antagonist noggin were also inhibited.

These results suggest that the activation of canonical wnt signaling by LiCl suppressed BMP4 protein synthesis or degraded BMP4 and inhibited chondrogenesis. The molecular system to explain this result has not been well understood. Cathepsin H (ctsh) which is previously reported to degrade BMP4 in the developmental stage of lung. The does-dependent upregulation of ctsh by LiCl was also seen in our RT-PCR examination.



Disclosures: ***K.N. Kishimoto****, None.*

SA157

Coordinate Regulation of PTHrP and PTH1R in the Osteoblast Differentiation of C2C12 Cells Promoted by BMP-2. A. R. G. Susperregui[1], F. Viñals*[1], P. W. M. Ho*[2], M. T. Gillespie[2], T. J. Martin[2], F. Ventura*[1]. [1]Barcelona University, Barcelona, Spain, [2]St. Vincent's Institute of Medical Research, Fitzroy, Australia.

PTH1R expression is enhanced during osteoblast differentiation and it is accepted as an osteoblast differentiation marker. Here we report that BMP-2 early induces PTH1R mRNA in C2C12 cells, which consequently become responsive to PTHrP in terms of cAMP production. Moreover, this induction is partially dependent on p38alpha as observed in experiments using p38alpha (-/-) MEFs. Simultaneously, BMP-2 also regulates the RANKL/OPG system strongly reducing the RANKL:OPG gene expression ratio. In our model, addition of exogenous PTHrP (1-34) does not modify BMP-2 regulation of RANKL and OPG. However, PTH1R expression and function affect osteoblast differentiation of C2C12 cells since PTHrP reduces the up-regulation of osteoblast master genes such us Runx2 and Osterix. Particularly PTHrP inhibits Osterix expression by activating the PKA pathway but not deregulating the Smad signaling pathway. C2C12 cells also modulate PTHrP expression in response to BMP-2. PTHrP mRNA expression is reduced by transcriptional regulation of PTHrP promoter but not enhancing PTHrP mRNA degradation. These data support the importance of a coordinate regulation of PTHrP and its receptor during osteoblastogenesis, and suggest that BMP-2 down-regulation of PTHrP could facilitate terminal differentiation of osteoblast.

Disclosures: ***A.R.G. Susperregui****, None.*

SA158

See Friday Plenary number F158.

SA159

Impaired Bone Healing in a BMP2 Knockout Mouse Model of Distraction Osteogenesis. N. Alam*[1], T. Haque*[2], M. Kotsiopriftis*[3], D. Lauzier*[2], R. St-Arnaud*[1], R. C. Hamdy[2], V. Rosen*[4]. [1]Genetics, Shriners Hospital for Children, Montreal, QC, Canada, [2]Orthopaedics, Shriners Hospital for Children, Montreal, QC, Canada, [3]Montreal Childrens Hospital, Montreal, QC, Canada, [4]Developmental Biology, Harvard School of Medicine, Boston, MA, USA.

Distraction osteogenesis (DO) is a surgical technique widely used for the treatment of limb length discrepancies, limb deformities, long bone non unions as well as bone loss due to trauma, infection or malignancies. One of its limitations is the long period of time required for the bone to consolidate. Bone Morphogenetic Proteins (BMPs), are essential growth factors involved in development (pre and post natal) and fracture healing. However, its specific role in DO remains to be determined. Modulating the signaling pathway of several BMPs including BMP 2, 4, 6 and 7 may be a potential strategy for accelerating bone formation during DO. The aim of the following study was to determine the significance of BMP2 during DO.

We analyzed bone formation during DO in heterozygous conditional BMP2 knockout mice. Bone samples were analyzed using MicroCT, Histology as well as Immunohistochemistry at various time intervals during the distraction and consolidation phase. Our results revealed that early bone consolidation is not impaired in mice having a 50% reduction in BMP2. MicroCT analysis showed no significant difference in bone volume, tissue volume or trabecular structure during the distraction phase and early consolidation phases. However, MicroCT images revealed poor bone healing at the end of consolidation in the mutant mice and a statistically significant reduction in trabecular number and increase in trabecular separation was apparent. Immunohistochemistry results showed that mutants had a higher upregulation of BMP2, BMP3, BMP7 BMPRIb and the activin receptor compared to the control during the distraction phase. However, during early consolidation, with the exception of BMPRIb, the controls had a higher expression of these genes.

In conclusion, our results indicated that in DO, BMP2 may be essential for proper bone healing at the end of consolidation but not during early consolidation. Several factors could explain these finding which include the possibility that during early bone consolidation, the function of BMP2 may be compensated for by other growth factors triggered by the process of distraction. There may also be a reserve of BMP2 within the bone, and thereby an endogenous reduction of its expression does not affect early bone healing. Further studies are being conducted in order to understand the complete role of BMP2 during DO in the molecular level.

***Disclosures:* N. Alam**, *None.*

This study received funding from: Shriners Grant.

SA160

The Expression of BMP Antagonists During Fracture Healing. D. B. Dean*, H. C. Peters*, J. T. Enders*, W. Jin*, J. T. Watson*, Z. Zhang*. Saint Louis University, St. Louis, MO, USA.

The bone morphogenetic protein (BMP) signalling cascade is “fine-tuned” by a group of BMP antagonists at almost every level of the pathway. This study investigated the temporal relationship between BMPs and their antagonists in bone formation using a normal fracture healing model.

Forty-two C57BL/6 mice (male, 7-week-old, approved by Saint Louis University Animal Care Committee) underwent controlled mid-shaft femoral fracture. Fractures were assessed with weekly radiographs, and fractures were healed in 3 weeks. Sequential sacrifice and tissue sampling of fracture callus were carried out at days 1, 3, 7, 14 and 21 for RNA isolation and immunohistochemistry. The tissue blocks were centered at the fracture line and included 2 fracture ends. Femoral bone segments from mid-shaft of the same aged unfractured mice were resected for experimental control. Real time RT-PCR was performed for the expression of BMP-2, -4, -6, -7; BMPR-1A, -2; and BMP antagonists: noggin, DAN, CHRD, BAMBI, PRODC, SOST, SMAD6, SMAD7 CERBERUS, and Grem1.

Genes of the BMP and BMP receptors: Most noticeable trend was the upregulation of the expression of BMP-2 and 7. Both were significantly increased at day 7 (3.27 and 2.83-fold, respectively), and gradually decreased in the 2nd and 3rd weeks of fracture healing. While the expression of BMP-4 was increased with no statistical significance, BMP-6 expression decreased except at day 7, when it was virtually the same as the control. BMPR-2 was statistically unchanged at most time-points. In contrast, the expression of BMPR-1A was decreased at the most time-points.

Genes of BMP antagonists: The expression of PRDC, SOST, SMAD7, GREM1 and CERBERUS was downregulated during the course of fracture healing, except that, at day 7, CERBERUS was expressed at the control level. In contrast, noggin was significantly upregulated from day 1 through day 7 and decreased its expression at days 14 and 21. Three other genes, DAN, CHRD and BAMBI, had increased expression only after day 7. While, the increase of expression of DAN and BAMBI continued to day 21, CHRD was downregulated at day 21.

Using a mouse fracture model, this study demonstrated the interactions between BMPs and their antagonists during the course of normal fracture healing. The alterations of gene expression during fracture healing of each of the studied BMP antagonist are evident of the participation of BMP antagonists to bone formation. The roles of BMP antagonists in fracture healing are as diverse as BMPs. Some of them are suppressed, which may facilitate releasing and activation of BMPs. Some, which are upregulated in the late stages of fracture healing, may contribute to the bone remodelling process.

***Disclosures:* Z. Zhang**, *None.*

This study received funding from: Foundation for Orthopaedic Trauma.

SA161

Dual Roles of Smad Proteins in the Conversion from Myoblasts to Osteoblastic Cells by BMPs. J. Nojima[1], K. Kanomata*[1], T. Fukuda[1], A. Nakamura[1], T. Tsukui*[2], Y. Okazaki*[3], R. Kamijo[4], T. Yoda*[5], T. Katagiri[1]. [1]Division of Pathophysiology, Saitama Medical University,Resarch Center for Genomic Medicine, Hidaka-shi,Saitama, Japan, [2]Division of Experimental Animal Laboratory, Saitama Medical University,Resarch Center for Genomic Medicine, Hidaka-shi,Saitama, Japan, [3]Division of Functional Genomics & System Research, Saitama Medical University,Resarch Center for Genomic Medicine, Hidaka-shi,Saitama, Japan, [4]Department of Biochemistry, School of Dentistry, Showa University, Sinagawa-ku, Tokyo, Japan, [5]Department of Oral & Maxillofacial Surgery, Saitama Medical University, Moroyama, Saitama, Japan.

(Objective) BMPs induce ectopic bone formation in muscle tissue in vivo and convert differentiation pathway of myoblasts to differentiate into osteoblastic cells in vitro. At the last ASBMR Annual Meeting, we have reported that a constitutively activated mutant Smad1, Smad1(DVD), induced ALP activity and Osterix mRNA in C2C12 myoblasts. Here, we further examined the molecular mechanisms of the conversion of myoblast differentiation by BMPs.

(Method and Result) Although wild-type or Smad1(DVD) shoed minimal inhibitory effect on myogenic differentiation, co-expression of Smad4 markedly reduced number of myosin heavy chain (MHC)-positive muscle cells. We found that the inhibition of myogenic differentiation was induced by nuclear Smad4 alone. The role of Smad4 in myogenic differentiation was confirmed using mouse embryonic fibroblasts (MEFs) prepared from $Smad4^{floxed/floxed}$ mice. The basal transcriptional activity of MyoD was increased approximately 1.4-fold in MEFs infected with Cre-expressing virus, and this increase was not suppressed by BMP-4. A deletion analysis of Smad suggested that MH2 domain may interact with other partner(s) to suppress the myogenic differentiation. We searched a protein-protein interaction (PPI) database which was constructed based on the mammalian two-hybrid method established by the RIKEN group, and found some Smad-4 binding proteins. Among them, we focused on E4F1, which contains zinc fingers and a ubiquitin E3 ligase domain. E4F1 was expressed in nuclei. The interaction between E4F1 and Smad4 was detected in nuclear Smad4 via its MH2 domain. Over-expression of wild-type E4F1 or a mutant protein lacking E3 ligase domain suppressed myogenic differentiation in the absence of BMPs. Knockdown of endogenous E4F1 reversed the inhibition of myogenic differentiation by BMP signaling.

(Conclusion) We found that Smad4 and E4F1, a novel partner of nuclear Smad4, cooperatively regulate the inhibition of myogenic differentiation by BMP signaling. Taken together, these findings suggest that the Smad signaling pathway may play dual roles in the conversion of myoblasts to osteoblastic cells induced by BMPs.

***Disclosures:* J. Nojima**, *None.*

SA162

Improvement of Alveolar Bone Quality by Local bFGF Injection - Histological and Cellular Biological Analysis in a Rabbit Model. M. Oshima*, W. Sonoyama, M. Ono*, K. Shimono*, T. Hikasa*, Y. Okamoto*, Y. Tsuchimoto*, Y. Matsuka*, T. Kuboki*. Department of Oral Rehabilitation and Regenerative Medicine, Okayama University, Okayama, Japan.

Basic fibroblast growth factor (bFGF) is known to have multiple roles in bone development and regeneration. Although it is already reported that bFGF accelerates the healing of periodontal bony defect and increases the bone quantity, its effect on local bone quality is not clarified yet. In this study, recombinant human bFGF was injected into bone marrow spaces of the rabbit mandible, then bone quantity and quality was examined histologically and bone morphometric parameters were analyzed by μCT. In addition, bone marrow cells were isolated, and the effects of bFGF on these cells were examined *in vitro* in terms of osteogenesis and osteoclastogenesis. Ilium and tibia was used as a control against mandible.

Four weeks after injection (100 μg of bFGF), accelerated bone formation was observed obviously in the cancellous bone area of the mandible, but not in the tibia. μCT analysis revealed that several parameters (i.e. bone volume/tissue volume, trabecular thickness, and trabecular number) were significantly improved only in the mandible against control (normal saline solution injected-site). Although MTS assay revealed that cell proliferation was enhanced dose-dependently by bFGF in all osteogenic cells (isolated from bone marrow of mandible, ilium, and tibia), proliferative capacity of the mandibular osteogenic cells was significantly higher than that of the iliac and tibial cells. Under an osteogenic induction condition, the tibial osteogenic cells showed higher calcified nodule formation than the others at day-7 after osteogenic induction. Osteogenesis was suppressed by bFGF in all osteogenic cells. Osteoclastgenesis, analyzed by TRAP staining, was suppressed by high-dose (10^{-9}M) bFGF, and enhanced by low-dose (10^{-12}M) bFGF in all bone marrow cells isolated from the mandible, ilium, and tibia. However, the number of mature multinucleated osteoclasts derived from the iliac and tibial bone marrow was more than that from the mandibular bone marrow. Bone resorption activity was evaluated by pit formation assay and found to be suppressed by high-dose (10^{-9}M) bFGF. Identical to the results of TRAP staining, bone resorption area made by the iliac and tibial osteoclasts was much larger than that by the mandibular osteoclasts.

These results suggest that local bFGF injection can be one of the suitable strategies to improve alveolar bone quality, possibly due to the different nature of the mandibular bone marrow.

***Disclosures:* M. Oshima**, *None.*

SA163

See Friday Plenary number F163.

SA164

NARS Induced by Basic Fibroblast Growth Factor Regulates the Proliferation and Survival of Osteoblasts. S. Park*[1], J. Byun*[2], H. Choi*[2], G. Jargalan*[1], Y. Rhee[2], S. Lim[2]. [1]Brain Korea 21 Project for Medical Science, College of Medicine, Yonsei University, Seoul, Republic of Korea, [2]Division of Endocrinology and Endocrine Research Institute, Department of Internal Medicine, College of Medicine, Yonsei University, Seoul, Republic of Korea.

Basic Fibroblast growth factor (bFGF), the potent bone anabolic agent, regulates bone development, growth, remodeling and fracture healing. The intracellular signaling of bFGF leads to activation of genes involved in cell proliferation, migration, differentiation and survival. However, little is known about bFGF-regulated proteins in bone. Therefore, in this study, protein profiling in bFGF-treated MC3T3-E1 preosteoblast cells was evaluated using proteomics. Asparaginyl-tRNA synthetase (NARS) was one of the proteins that up-regulated more than ten-fold in MC3T3-E1 cells after treatment of bFGF. NARS is a member of aminoacyl-tRNA synthetases (ARSs) and is responsible for catalyzing the specific aminoacylation of tRNA with asparagines. Overexpression of NARS significantly increased the level of proliferation and conferred resistance to apoptosis induced by serum deprivation in MC3T3-E1 cells and calvarial cells. In contrast, the level of proliferation was remarkably decreased and cell death was increased in siNARS-trnasfected cells compared to those of control siRNA-transfected cells. In conclusion, NARS induced by growth factors could be one of highly linked enzymes to the proliferation and survival of osteoblastic cells.

***Disclosures:* S. Park**, *None.*

SA165

Serum FGF-23 Level in Term Infants. M. Takaiwa[1], K. Aya*[2], T. Miyai*[2], B. Yuan[3], M. Yokoyama*[4], M. K. Drezner[3], N. Kodani*[1], T. Morishima*[2], H. Tanaka[5]. [1]Dept. of Pediatrics, Matsuyama Red Cross Hospital, Matsuyama, Japan, [2]Dept. of Pediatrics, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan, [3]Dept. of Medicine, University of Wisconsin, Madison; GRECC, Wm F. Middleton VAMC, Madison, WI, USA, [4]Dept. of Obstetrics and Gynecology, Matsuyama Red Cross Hospital, Matsuyama, Japan, [5]Dept. of Pediatrics, Okayama Saiseikai General Hospital, Okayama, Japan.

Recent studies demonstrated that FGF-23 (fibroblast growth factor 23) is not only crucial in the pathogenesis of inherited and acquired hypophosphatemia, but also a physiological regulator of Ca and P homeostasis. Hence, serum FGF-23 level would be widely applied as a clinical marker for assessment of mineral metabolism. In our previous study on mouse FGF-23, we reported both serum level and mRNA expression of FGF-23 are elevated in pre-weaned mice than that of mature animals. From these results, we decided to establish the normal range of human serum FGF-23 level during neonatal period to utilize FGF-23 for assessment of mineral metabolisms during infancy. In this study, we examined serum FGF-23 level at 5 days after birth of normal term neonates (5 day-old infants, N=8). We also analyzed serum from umbilical vain (cord blood, N=6). As control, we tested serum of healthy adults (healthy adults, N=6). As known, Ca level in normal newborns falls rapidly within first 48 hours of life (early neonatal hypocalcemia) and consecutively, intact PTH (iPTH) and 1,25(OH)2D3 (1,25D)start elevating. Thus, we also measured serum Ca, P, iPTH and 1,25D level. All protocols were approved by the Matsuyama Red Cross hospital ethical committee. Informed consent was obtained from all participants or legal guardians. FGF-23 level was measured using FGF-23 ELISA kit (Kainos). Data are expressed as the mean ± SEM and statistically analyzed by a 'Bonferroni' testing. A value of $p<0.05$ was considered significant.
Serum FGF-23 level is significantly elevated in 5 day-old infants (65.8 +/- 10.1 pg/ml) than in healthy adults (32.0 +/- 8.0 pg/ml, $p<0.05$). On the other hand, serum FGF-23 level was not different from healthy adults in cord blood samples (22.6 +/- 4.9pg/ml). Serum Ca, P, iPTH and 1,25D levels were compatible with previous reports. Considering P is a potent regulator of FGF-23 expression, and our previous study indicated that the elevated serum FGF-23 level in murine infants is due to increased FGF-23 mRNA expression, our present observations indicate that FGF-23 is induced in response to hyperphosphatemia commonly accompanied with early neonatal hypocalcemia. Although further investigations are required, our results suggested that regulatory mechanism of FGF-23 is already active, and may have considerable contribution in mineral metabolism during early infancy.

***Disclosures:* M. Takaiwa**, *None.*

SA166

IL-12 Induces Apoptosis in TNF-α-mediated Osteoclastogenesis *in vivo*. M. Yoshimatsu, H. Kitaura, Y. Fujimura*, T. Eguchi*, H. Kohara, Y. Morita*, N. Yoshida*. Division of Orthodontics and Dentofacial Orthopedics, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan.

Recently, much importance has been placed on the relationship between cytokines and bone metabolism. We reported that IL-12 induced apoptosis in bone marrow cells treated with TNF-α resulted in the inhibition of osteoclastogenesis *in vitro*. This study aimed to investigate the effects of IL-12 on TNF-α osteoclastogenesis *in vivo*. C57BL6/J mice (8-week-old males) were classified into three groups. Each group was subjected to 5 daily supracalvarial administrations of TNF-α as a positive control, TNF-α and IL-12, or PBS as a negative control. Animals were sacrificed on day 6 and fixation and demineralization performed. Histological sections of calvariae were stained for tartrate-resistant acid phosphatase (TRAP) and TUNEL. For isolating total RNA, mice calvaliae frozen in liquid nitrogen were homogenized. mRNA was isolated from homogenized samples using the TRIzol reagent. cDNA was synthesized from total RNA. The expression levels of Cathepsin K, TRAP, Fas and FasL mRNA of mice calvariae were quantified by real-time-based RT-PCR. Many osteoclasts were observed in mice administered TNF-α. On the other hand, the numbers of osteoclasts in mice administered TNF-α and IL-12 were less than those treated with TNF-α. The expression levels of Cathepsin K and TRAP were also reduced in mice administered TNF-α and IL-12 compared with mice administered TNF-α. The mRNA level of Fas was increased when TNF-α was administered and the mRNA level of FasL was increased when IL-12 was administered. The apoptotic alteration of calvalial cells in suture was recognized when TNF-α and IL-12 were administered. In this study, IL-12 was shown to inhibit TNF-α-mediated osteoclastogenesis by apoptosis *in vivo*. Apoptotic alteration might be induced by the interaction of TNF-α-induced Fas and IL-12-induced FasL.

***Disclosures:* M. Yoshimatsu**, *None.*

SA167

A Model of High Body Mass: Bone Mineral Density, Bone Markers and Adipocytokine Levels in High Level Male Rugby Players. S. Breban*, C. Chappard, C. Benhamou. CHR Orleans, INSERM Unit 658, Orleans, France.

Rugby practice offers a chance of studying very high body mass subjects and the repercussion on BMD measurements, bone metabolism and adipocytokine concentrations. Forty six men aged between 17 and 28 years participated in this study and were divided in two groups: 20 national or international ranked rugby players (aged 21.6±4.6 years; 11.9±2.9 hours/week) and 26 age and sex matched controls (aged 22.7±4.5 years; 2.2±2.0 hours/week). BMC (g) and BMD (g/cm²) were measured by DXA (Delphi, Hologic ®, Waltham, MA), at whole body (WB), lumbar spine (L1-L4) and non dominant femoral neck (FN). Height, weight and body composition (whole body lean mass (kg) and fat mass (kg)) were derived from the WB DXA measurements. Circulating levels of serum leptin (ng/mL) and adiponectin (µg/mL), serum osteocalcin (ng/mL) and C-terminal telopeptides of type I collagen (Ctx) (ng/mL) were assessed by ELISA assay. Rugby players had significantly higher BMD values at WB ($p<0.0001$), L1-L4 ($p<0.0001$) and non dominant FN ($p<0.0001$) compared to controls. These differences were maintained after adjustment to height, adipocytokine concentration and bone markers concentration, but there were no more differences when BMD values were adjusted to weight and WB lean mass. Rugby players also presented significantly higher concentrations of oesteocalcin ($p<0.001$) and Ctx ($p=0.006$). Leptin concentrations were significantly lower in athletes ($p=0.02$) and this persisted after adjustment to WB fat mass. However, there was no significant difference for adiponectin concentrations between athletes and controls. No significant relationships were found between leptin concentrations and any measured BMD parameters in rugby players and controls ($p>0.05$). Male rugby players seemed to present a specific bone metabolism with very high BMD and bone turnover markers values. These BMD levels were as a majority explained by a higher WB lean mass in athletes compared to controls. Our results also suggested that, high body mass was associated with lower serum leptin levels. Finally, leptin concentrations were not directly related to BMD measurements in young adults.

***Disclosures:* S. Breban**, *None.*

SA168

See Friday Plenary number F168.

SA169

Mesenchymal Stem Cells Enhance Fracture Healing: Essential Role for Cytokines in Homing and Anti-Inflammatory Response. F. Granero-Molto[1], J. A. Weis*[1], B. Landis*[2], L. Longobardi*[1], M. I. Miga*[3], A. Spagnoli[1]. [1]Pediatric Endocrinology, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, [2]Pediatric Endocrinology, Vanderbilt University, Nashville, TN, USA, [3]Biomedical Engineering, Vanderbilt University, Nashville, TN, USA.

Healing failure occurs in 10-20% of the bone fractures. Limitations in adult stem cells (MSC) have been shown to be a key element in lack of healing. In this study, we determine: 1) an essential homing receptor for the migration of MSC to the fracture site; 2) the systemic anti-inflammatory effects of MSC; 3) the MSC regenerative role. MSC were

isolated from 4-6 week old syngenic FVB males mice constitutively expressing luciferase. Syngenic FVB female mice 8-10 weeks old were subjected to a three point bending stabilized fracture and injected with 10^6 MSC. Fractured females without transplant were used as control (NC). The anti-inflammatory effect of MSC was analyzed detecting levels of TNFα and IL-1β in the serum of MSC and NC animals at 1, 3 and 7 days post-fracture (PF). Healing was assessed by distraction biomechanical testing (BMT) at 14 days. For migration studies, MSC were selected based on the presence or absence of the surface receptor CXCR4. The *in vivo* MSC migration was detected using bioluminescence at 1, 3, 7 and 14 days PF and their influence on the callus properties analyzed by μCT.
BLI studies showed that MSC migrated to the fracture site and CXCR4 was essential for MSC homing. In fact, in mice transplanted with MSC lacking CXCR4, cell homing to the fracture was totally abolished. Furthermore, CXCR4 transplant produced a significant increase in the total and soft tissue volume of the callus (Fig. 1). Transplanted MSC reduced the serum levels of the pro inflammatory cytokines TNFα and IL-1β at day 1 and 3 PF.

*p<0.05 vs NC		Day 1 PF	Day 3 PF	Day 7 PF
TNFα	NC (n=5)	89.44 ± 39.97	51.29 ± 27.19	6.69 ± 3.32
(pg/ml)	MSC (n=6)	17.68 ± 6.82*	9.57 ± 5.08*	9.13 ± 4.85
IL-1β	NC (n=5)	84.16 ± 44.23	40.48 ± 5.03	8.25 ± 10.04
(pg/ml)	MSC (n=6)	2.88 ± 1.81*	4.62 ± 3.93*	3.58 ± 2.66

BMT analysis showed that MSC transplant improved the healing by a significant increase in the energy to failure (NC, 0.14±0.05 mJ; MSC 0.49±0.11 mJ, p<0.05).
In conclusion, MSC have an anti-inflammatory effect, MSC migration is dependent on CXCR4 expression and the migration is beneficial for the fracture healing process.

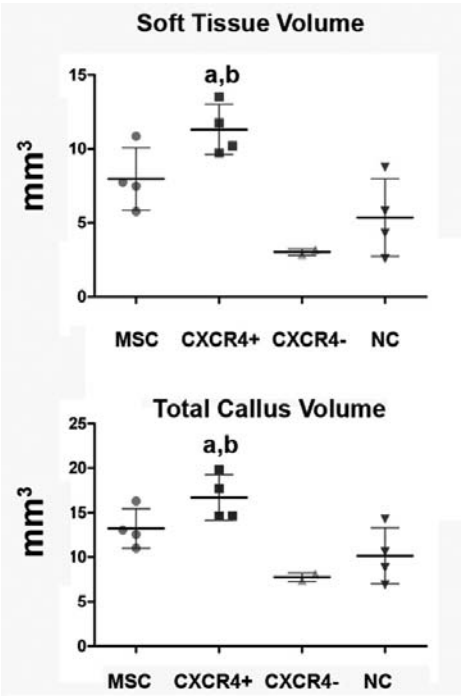


Figure 1: μCT analysis of the callus 14 days after fracture shows increased amount of soft tissue and total callus volume in CXCR4+ transplanted animals. a, p<0.05 CXCR4+ vs CXCR4-; b, p<0.05 CXCR4+ vs NC.

***Disclosures:** **F. Granero-Molto**, None.*
This study received funding from: NIH-NIDDK.

SA170

Transient Expression of CXC Receptor 2 in Human Mesenchymal Stem Cells Stimulates Chemotaxis Toward CXC Ligand 8 and Increased Mineralization in the Presence of Osteogenic Medium. D. S. Bischoff*, N. S. Makhijani*, D. T. Yamaguchi. Research Service, VA Greater Los Angeles Healthcare System, Los Angeles, CA, USA.

The potential role of CXC chemokines bearing the glu-leu-arg (ELR^+) motif in bone differentiation was studied using a human mesenchymal stem cell (hMSC) model. Previously, we have shown that osteogenic differentiation of hMSCs increases expression of the ELR^+ CXC chemokine CXC Ligand 8 (CXCL8), also known as Interleukin-8 or IL-8. hMSCs from various donors were tested and in only one case did the addition of exogenous CXCL8 (10 nM) induce the expression of the bone marker alkaline phosphatase (ALP). There are two ELR^+ CXC chemokines receptors, CXC Receptor 1 (CXCR1) and CXC Receptor 2 (CXCR2). CXCL8 binds equally well to CXCR1 and CXCR2 while the other ELR^+ CXC chemokines bind with much greater affinity to CXCR2. CXCR2 has been shown to be associated with the angiogenic function of the ELR^+ CXC chemokines. Since the levels of CXCR1 and CXCR2 vary among donors and seem to decrease with passage of the cells, we used the Amaxa Nucleofector Device to transiently transfect hMSCs with CXCR1 and CXCR2 expression plasmids or with the pTA vector as a negative control. Transfected cells were tested for the ability to migrate toward increasing levels of CXCL8 or CXCL1 (GROα) ranging from 0.1 to 100 nM using Transwell inserts. Non-transfected cells did not migrate even at the highest level of CXCL8 or CXCL1 (100 nM). Cells transfected with the negative control pTA did not migrate in response to 10 nM CXCL8. Those cells expressing CXCR1 had decreased levels of cell migration with a chemotaxis index (CI) value of 0.75 (ratio of migrating cells towards chemoattractant / migrating cells in negative control), and those expressing CXCR2 had increased levels of migration toward 10 nM CXCL8 with a CI value 1.9. Transfected hMSCs were also tested for the ability to induce mineral deposition by alizarin red staining. Cells expressing CXCR2 have larger mineralized nodules at 2 and 3 weeks post-transfection when grown in the presence of osteogenic medium (OGM) compared to cells transfected with either the pTA negative control or the CXCR1 expression plasmid. Expression of ALP mRNA was also induced at 7 days in the CXCR2-transfected cells in the presence of OGM. Transfection of pTA or the CXCR1 expression plasmid did not induce mineralization or ALP expression at 7 days in OGM. Cells grown in basal proliferation medium did not induce mineralization or ALP mRNA expression under any circumstances even in the presence of 10 nM CXCL8. Conclusion: 1) expression of CXCR2 in hMSC confers chemotaxic ability of hMSC cells toward CXCL8 and 2) stimulates expression of ALP mRNA and mineralization in the presence of OGM.

***Disclosures:** **D.S. Bischoff**, None.*
This study received funding from: VA Merit Review.

SA171

See Friday Plenary number F171.

SA172

Transforming Growth Factor β Receptor I Kinase Inhibitor Increases Bone Mass in Normal Mice. K. S. Mohammad[1], G. Balooch*[2], C. Chen*[2], E. Stebbins*[3], D. Wong*[3], R. Derynck*[2], L. Suva[4], T. A. Guise[1], T. Alliston[2]. [1]University of Virginia, Charlottesville, VA, USA, [2]University of California San Francisco, San Francisco, CA, USA, [3]Scios Inc., Fremont, CA, USA, [4]University of Arkansas for Medical Sciences, Little Rock, AR, USA.

TGFβ can promote and inhibit specific stages of osteoblast and osteoclast differentiation and function throughout development. Consequently, the net effect of altered TGFβ signaling on bone is difficult to predict, an uncertainty supported by the complicated bone phenotypes of mice with modified TGFβ signaling. Whether or not TGFβ can be modulated in a therapeutically beneficial way in bone remains unknown. Our recent findings show that partial inhibition of TGFβ signaling in genetically modified mice increases bone mass, bone matrix material properties such as elastic modulus and hardness, and fracture toughness. In addition, pharmacologic blockade of TGFβ signaling with SD-208, a specific inhibitor of the type I TGFβ receptor (TβRI), increases bone mass in mammary tumor bearing mice. Therefore, we sought to determine the effect of pharmacologic TβRI inhibition on the biological and mechanical properties of bone in normal mice.
C57/Bl6 mice were administered SD-208 (20 mg/kg or 60 mg/kg) or vehicle for 6 weeks (n=15/group). Bone mineral density (BMD) was assessed longitudinally by DXA and terminally by micro-CT and X-ray tomographic microscopy (XTM). TβRI inhibitors significantly increase BMD in male and female mice at all sites (whole body, spine, tibia, femur). MicroCT analysis showed that, trabecular, but not cortical, bone volume is increased by SD-208. Histomorphometry and in vitro differentiation assays revealed that TβRI inhibitors increase TBV by anabolic and anti-catabolic mechanisms. Osteoblast numbers and the bone formation rate are increased in SD-208-treated mice relative to vehicle treated controls, whereas osteoclast numbers are reduced. Likewise, bone marrow cultures from SD-208-treated mice have increased osteoblast colony forming activity, but a decreased capacity to form TRAP-positive multinucleated osteoclasts. Consistent with results in genetically modified mice, pharmacologic inhibition of TGFβ signaling increases the mineral concentration and elastic modulus of bone matrix, when measured by XTM and nanoindentation, respectively. Finally, vertebral bone from SD-208-treated mice exhibits an increased load to failure in unconfined compression tests. In conclusion, inhibition of TGFβ type I receptor function exerts both anabolic and anticatabolic effects on bone, causing a net increase in bone mass and vertebral bone load to failure. TGFβ inhibitors may have therapeutic value for treating diseases associated with low bone mass.

***Disclosures:** **K.S. Mohammad**, None.*

SA173

IL-12 Stimulates the Osteoclast Inhibitory Peptide-1 (OIP-1) Gene Expression in CD4$^+$ T-Cells. S. Shanmugarajan[1], N. Kawanabe[2], M. Koide[2], J. E. Arroyo*[1], L. L. Key[1], S. V. Reddy[1]. [1]Charles P. Darby Children's Research Institute, Medical University of South Carolina, Charleston, SC, USA, [2]Medicine-Hematology/Oncology, University of Pittsburgh, Pittsburgh, PA, USA.

Osteoclast formation and activity is regulated by local factors produced in the bone microenvironment. Immune cell products such as IFN-γ and IL-12 are potent inhibitors of osteoclast formation. We have previously identified and characterized the human osteoclast inhibitory peptide-1 (OIP-1/hSca), a member of Ly-6 gene family. Also, demonstrated that IFN-γ stimulates OIP-1 promoter activity and expression in osteoclast precursor cells. However, it is unknown if IL-12 regulates OIP-1 expression in the bone microenvironment. Real-time PCR analysis demonstrated that IL-12 treatment (4 hr) significantly enhanced OIP-1 mRNA expression in normal human bone marrow derived mononuclear cells. Since IL-12 induces IFN-γ production by T-cells, we further tested if IFN-γ participates in IL-12 stimulation of OIP-1 expression in these cells. IL-12 treatment in the presence of a neutralizing antibody against IFN-γ significantly increased OIP-1 mRNA expression, suggesting that IL-12 directly regulates OIP-1 gene expression. Interestingly, real-time PCR analysis demonstrated that IL-12 induces OIP-1 expression (3.2-fold) in CD4 positive T-cells; however, there was no significant change in CD8 positive T-cells. Also, IL-12 (10ng/ml) treatment of Jurkat T-cells transfected with OIP-1 gene (-1 to -1988 bp) promoter-luciferase reporter plasmid demonstrated a 5-fold and 2.7-fold increase in OIP-1 gene promoter activity in the presence and absence of antibody against IFN-γ, respectively. We previously identified a Stat binding motif (-1629 to -1639 bp position) in the OIP-1 gene promoter region. We further show that Stat-3 inhibitor peptide treatment decreased (42.8%) IL-12 stimulated OIP-1 promoter activity. In contrast, Stat-1 inhibitor has no significant effect on OIP-1 gene promoter activity. Chromatin Immunoprecipitation (ChIP) assay confirmed Stat-3, but not Stat-1 binding to the OIP-1 gene promoter element in response to IL-12 stimulation. These results suggest that IL-12 stimulates the OIP-1 gene expression through Stat-3 signaling in CD4 positive T-cells and that OIP-1 is an important autocrine/paracrine regulator of osteoclast development and bone remodeling.

Disclosures: ***S. Shanmugarajan****, None.*
This study received funding from: NIH.

SA174

See Friday Plenary number F174.

SA175

The Mechanism of Action of Lactoferrin's Bone Anabolic Activity. J. Cornish, A. Chhana*, J. M. Lin*, K. E. Callon*, I. R. Reid, D. Naot. Medicine, University of Auckland, Auckland, New Zealand.

Lactoferrin, an 80kDa iron-binding glycoprotein present in milk and other exocrine secretions in mammals, is anabolic to bone at physiological concentrations. Lactoferrin stimulates the proliferation, differentiation and survival of the osteoblasts, as well as potently inhibiting osteoclastogenesis in bone marrow cultures. In vivo, local injection of lactoferrin results in substantial increases in bone formation and bone area. In a critical bone defect model in vivo, lactoferrin was also seen to promote bone growth. The mitogenic effect of lactoferrin in osteoblast-like cells is mediated mainly through LRP1, a member of the low density lipoprotein receptor-related proteins that are primarily known as endocytic receptors; however another yet unidentified receptor is responsible for the anti-apoptotic actions. Lactoferrin also induces activation of multiple pathways: p42/44 MAPK signalling as well as PI3-kinase-dependent phosphorylation of Akt in osteoblasts.
Recently, we have further delineated the possible mechanisms of action of lactoferrin in bone, in both osteoclasts and osteoblasts. The mouse macrophage cell line, RAW 264.7, demonstrated that lactoferrin acts directly on osteoclasts to inhibit their development independently of osteoblasts. In addition, the receptor involved is *not* LRP1. In human osteoblasts treated with lactoferrin, we identified differentially-expressing genes, using microarray analysis then validated in real time PCR significant up-regulation of IGF1 and down-regulation of DKK1. We then extended these studies looking at earlier time points in the MC3T3-E1 osteoblastic cell-line, using real-time PCR with custom-designed microfluidic cards. The cards were used to measure the relative expression levels of 48 genes including bone-specific transcription factors, matrix and inflammatory proteins. Lactoferrin induced a rapid, dose-dependent increase in the transcription levels of IL-6, IL-11, the pro-inflammatory factor prostaglandin-endoperoxide synthase 2 (COX-2) and the transcription factor nuclear factor of activated T cells-1 (NFATc1). Subsequent real-time PCR experiments in primary rat osteoblasts confirmed that IL-6, IL-11, COX-2 and NFATc1 transcription levels increase significantly and transiently within two hours of lactoferrin treatment. The role these genes play in mediating the effects of lactoferrin in osteoblasts is currently being investigated by the use of specific inhibitors.
Lactoferrin is proving to be a positive regulator of bone growth potentially having a physiological role in bone growth and healing, and a therapeutic role as an anabolic factor in osteoporosis.

Disclosures: ***J. Cornish****, None.*
This study received funding from: Health Research Council of New Zealand.

SA176

See Friday Plenary number F176.

SA177

Stimulation of Macrophage TNFα Production by Orthopaedic Wear Particles Requires Activation of the ERK1/2/Egr-1 Pathway but Is Independent of p38 and JNK. M. A. Beidelschies*[1], H. Huang*[2], M. R. McMullen*[2], M. V. Smith*[1], A. S. Islam*[1], V. M. Goldberg*[1], X. Chen*[1], L. E. Nagy*[2], E. M. Greenfield[1]. [1]Orthopaedics, Case Western Reserve University, Cleveland, OH, USA, [2]Pathobiology, Cleveland Clinic Foundation, Cleveland, OH, USA.

Bone loss that causes aseptic loosening of orthopaedic implants is initiated by pro-inflammatory cytokines produced by macrophages in response to implant-derived wear particles. MAPK signaling pathways are activated by the particles; however, it is not clear which of the MAPK pathways are important for the initial response to the wear particles and which are only involved at later steps in the process, such as osteoclast differentiation. Here, we show that the ERK1/2, p38, and JNK pathways are rapidly activated by the wear particles. However, incubation of RAW264.7 cells or bone marrow-derived macrophages with specific inhibitors and their inactive analogues showed that ERK1/2 is required for the initial responses to the wear particles (TNFα mRNA expression and TNFα protein secretion). In contrast, the p38 and JNK pathways were not required for these responses. ERK1/2 activation by wear particles is also required for increased expression of the transcription factor Egr-1 as well as for Egr-1's ability to bind to the TNFα promoter. Measurements of luciferase activity in RAW264.7 macrophages transfected with TNFα promoter-luciferase constructs containing specific deletions and mutations demonstrated that Egr-1 is also required for wear particles to increase TNFα transcription. These results, together with our previous studies of the PI3K/Akt pathway, demonstrate that wear particles coordinately activate multiple signaling pathways and multiple transcription factors to stimulate production of pro-inflammatory cytokines, such as TNFα. The current study also demonstrates that the ERK1/2/Egr-1 signaling pathway is activated to a much greater extent by wear particles with adherent endotoxin than by "endotoxin-free" wear particles. These results demonstrate that the ERK1/2/Egr-1 pathway contributes to the ability of adherent endotoxin to potentiate cytokine production, osteoclast differentiation, and bone loss induced by wear particles.

Disclosures: ***M.A. Beidelschies****, None.*

SA178

Alterations in Bone Structure and Growth in Dwarf Rats with a Depressed Growth Hormone/IGF-I Axis. A. C. F. Bassit, M. K. Altman*, S. E. Franz*, M. E. Leal, T. J. Wronski. Physiological Sciences, University of Florida, Gainesville, FL, USA.

Growth hormone (GH) is an important factor in the regulation of bone growth and the maintenance of adult bone. The majority of its skeletal effects appear to be mediated by insulin-like growth factor I (IGF-I). The dwarf rat is an animal model for studies of the effects of GH/IGF-I deficiency on bone structure and function. In these mutant rats, GH synthesis is selectively reduced to about 6% of normal in females, and serum IGF-I levels are about 10% of normal. Body growth is retarded, but the dwarf rats are healthy and skeletal malformations do not occur. The objective of this study was to use imaging (pQCT) and histomorphometric techniques to determine the skeletal consequences of GH/IGF-I deficiency in dwarf rats. Groups of normal Lewis rats (N=7) and dwarf rats (N=7) were maintained in the same nutritional and environmental conditions until 11 weeks of age. At the time of euthanasia, the mean body weight of the Lewis rats was 52.9% higher than the dwarf rats (187.6 ± 10.9g vs. 122.7 ± 5.6g, P<0.0001), and their left femurs were 14.8% longer. Not surprisingly, tibial longitudinal bone growth was three-fold greater in the Lewis rats compared to the dwarf rats (72.1 ± 4.9 μm/d vs. 24.5 ± 10.7 μm/d, P<0.0001). All the bone structural parameters evaluated by pQCT in the femur, including total mineral content, total mineral density, trabecular content, trabecular density, trabecular area, cortical density, cortical area, cortical content, cortical thickness, and periosteal circumference, were significantly greater in the Lewis rats compared with the dwarf rats. Cancellous bone histomorphometry was performed in the right proximal tibia. Cancellous bone volume was markedly lower in the dwarf rats than in the Lewis rats (1.2 ± 1.4% vs. 18.9 ± 3.3%, P<0.0001), and this cancellous osteopenia was associated with decreased trabecular number and width, and increased trabecular separation. Mean values for osteoclast surface, an index of bone resorption, were nearly identical in Lewis and dwarf rats. Although mean values for osteoblast and osteoid surfaces were not significantly different between the two groups, cancellous mineral apposition rate, an index of osteoblast activity, was significantly lower in dwarf rats compared with Lewis rats (0.9 ± 0.2 μm/d vs. 1.5 ± 0.3 μm/d, P<0.0005). These findings indicate that GH/IGF-I deficiency in dwarf rats has profound negative effects on bone growth, accumulation of bone mass, and osteoblast activity. In view of the contention that IGF-I may mediate the skeletal effects of bone anabolic agents, dwarf rats appear to be a promising animal model for studies of these interactions.

Disclosures: ***A.C.F. Bassit****, None.*
This study received funding from: NIH Grant R37 AG09241.

SA179

IGF-I Secreted from Osteoblasts as a Major Chemotactic Factor for Osteoblasts. M. Nakasaki[1], K. Yoshioka*[1], H. Yoshikawa*[2], K. Itoh[1]. [1]Biology, Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka, Japan, [2]Orthopedic Surgery, Osaka University Medical School, Osaka, Japan.

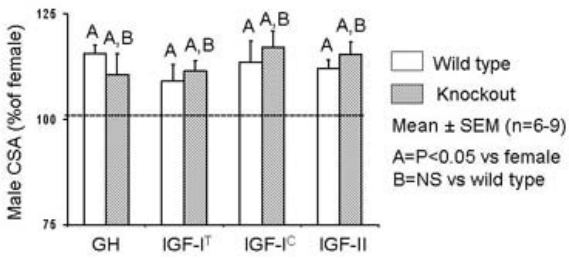
Osteoblast recruitment to the site of future bone formation is essential for skeletal development, bone remodeling and fracture healing, but the mechanism of which remains to be clarified. Here, we hypothesized that osteoblasts secrete a chemoattractant(s) for osteoblast recruitment and examined the serum-free conditioned medium of mouse osteoblast-like cell line MC3T3-E1 for its ability to induce osteoblast migration. Using a modified Boyden chamber assay, we found that the medium induced MC3T3-E1 chemotaxis in a dose-dependent manner. Employing several chromatographic procedures and liquid chromatography equipped with tandem mass spectrometry analysis, we identified insulin-like growth factor-I (IGF-I) as a potent chemotactic factor from the conditioned medium. IGF-I induced cell migration of both MC3T3-E1 cells and primary mouse osteoblasts, and checkerboard analysis revealed that IGF-I induced chemotaxis and not simply chemokinesis. By neutralization of IGF-I activity with specific anti-mouse IGF-I antibody, both osteoblast monolayer wound healing and cellular polarization were impaired, whereas human IGF-I replenishment reversed these inhibitory effects. IGF-I also promoted cell spreading on fibronectin in an integrin beta1-dependent manner. IGF-I induced Akt and ERK phosphorylation in MC3T3-E1 cells and a PI3K inhibitor, LY294002, but not a MEK inhibitor, PD98059, inhibited IGF-I-induced cell migration and wound healing. Together, these findings suggest that IGF-I secreted from osteoblasts regulates osteoblast migration through the activation of PI3K signaling.

***Disclosures:* M. Nakasaki**, *None.*

SA180

Is Growth Hormone/IGF-I Mediated Mechanism Involved in Regulating Gender Differences in Bone Size? S. Mohan, K. E. Govoni, J. E. Wergedal. JLP VA Medical Center and Loma Linda Univ, Loma Linda, CA, USA.

It is now generally well accepted that estrogen and testosterone exert opposite effects on periosteal bone expansion and that greater bone size in males is mediated via androgens acting through androgen receptors. However, the molecular pathway by which estrogens and androgens exert gender-specific effects on periosteal expansion remains to be fully elucidated. Based on the findings that growth hormone (GH)/IGF-I is a major regulator of postnatal skeletal growth, we and others have predicted that sex hormones interact with GH/IGF axis to regulate bone size. To evaluate this hypothesis, we compared femur cross sectional area (CSA) measured by pQCT in transgenic mice with disruption of GH/IGF axis. We predicted if gender differences in bone size are due to interaction between sex hormone and GH/IGF axis, then disruption of GH/IGF axis should eliminate gender differences in bone size. GH-deficient lit/lit mice with mutation in GH releasing hormone receptor, IGF-I total knockout (IGF-I^{T}, lacks both endocrine and local IGF-I), osteoblast specific IGF-I conditional knockout (IGF-I^{C}, lacks osteoblast produced local IGF-I) and IGF-II null mice were evaluated at 3 and 8 weeks of age. Corresponding age-matched control littermate mice were used as wild types. As expected, at prepubertal age of 3-weeks, femur CSA was not different between male and female mice for any of the strains studied.



At post pubertal age of 8 weeks, femur CSA was significantly greater in the males compared to females for all four transgenic lines studied at 8 weeks of age. To our surprise, disruption of GH, IGF-I, osteoblast-derived IGF-I or IGF-II did not affect bone size differences between male and female mice at 8 weeks (Figure). However, femur CSA was decreased as previously reported by 39, 67, 31 and 21% respectively in mice lacking GH, total IGF-I, osteoblast-derived IGF-I or total IGF-II respectively, thus demonstrating the importance of GH/IGF axis in regulating bone size during postnatal growth. In conclusion, our data using the transgenic mouse models do not support the hypothesis that GH/IGF axis is a major player in contributing to gender specific effect on bone size and suggest that the greater bone size in male mice is due to GH/IGF-independent actions of androgen on bone.

***Disclosures:* S. Mohan**, *None.*
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SA181

See Friday Plenary number F181.

SA182

Insulin-Like Growth Factor Binding Protein (IGFBP)-6 Is a Negative Regulator of Osteoblast Differentiation and Bone Formation. C. A. Strohbach[1], C. H. Rundle[2], J. E. Wergedal*[2], T. A. Linkhart*[2], S. Mohan[2], D. D. Strong*[2]. [1]Loma Linda University, Loma Linda, CA, USA, [2]MDC, J.L.P. VAMC, Loma Linda, CA, USA.

We previously reported that IGFBP-6 (BP6) is a negative regulator of osteoblast (Ob) differentiation, with BP6 retroviral overexpression inhibiting MC3T3-E1 mineralization and Ob marker gene expression possibly via an IGF-independent intracellular mechanism. To further elucidate the role of BP6 in Ob differentiation, we used siRNA to knockdown BP6 mRNA and protein in MC3T3-E1 pre-Obs and less differentiated C2C12 cells and observed an increase in Osterix, Dlx5, ALP, BSP, Osteocalcin and Phex mRNAs. While our *in vitro* studies provide compelling evidence that BP6 can play an important inhibitory role in Ob differentiation, the role of BP6 in normal skeletal development and maintenance has not been established. To address this question, transgenic (Tg) mice were generated that overexpressed BP6 in Obs. The BP6 transgene was placed under the control of an Ob lineage restricted promoter that contained the proximal Col1a2 promoter and modified Runx2 enhancer sequences. Col1a2 and Runx2 are early marker genes for Ob lineage commitment and this chimeric promoter was not suppressed by BP6 transgene overexpression. Multiple founders were identified and backcrossed two generations to reduce effects of genetic background differences and establish independent Tg lines. BP6 was expressed in bones isolated from BP6 Tg mice but not in other tissues. Skeletal parameters were studied by DEXA in BP6 Tg and wild type littermate controls at 6, 12, and 24wks. Areal BMD was significantly reduced in the femurs of male (7%, $p < 0.03$, n=8) and female mice (14%, $p < 0.002$, n=8) in two independent lines of BP6 Tg mice compared to wild-type littermate controls providing evidence that BP6 is a negative regulator of bone formation in vivo. However, the BP6 Tg mice did not display differences in body weight or femur length, suggesting that BP6 does not influence the growth promoting effects of the IGFs that have been observed in transgenic mice expressing other IGFBPs. In conclusion, our previous and current findings demonstrate that BP6 is an important negative regulator of Ob differentiation and bone formation and that BP6 effects on Obs involve decreased expression of transcription factors and marker genes that are critical for Ob differentiation.

***Disclosures:* C.A. Strohbach**, *None.*
This study received funding from: VA Merit Award.

SA183

Serum IGF-1 Is a Developmental Determinant of Bone Size. S. Yakar[1], E. Canalis[2], W. Mejia*[1], H. Sun*[1], Y. Wu*[1], Y. Kawashima*[1], P. Nasser*[3], V. Williams*[3], K. J. Jepsen[3]. [1]Endocrinology, Mount Sinai School of Medicine, New York, NY, USA, [2]St. Francis Hospital and Medical Center, Hartford, CT, USA, [3]Orthopaedics, Mount Sinai School of Medicine, New York, NY, USA.

Serum insulin-like growth factor I (IGF-I) originates mainly from the liver. Liver-specific *igf1* gene deletion (LID mouse model) causes abrogated expression of IGF-1 mRNA in liver, dramatic reductions (75%) in circulating IGF-1 and a 3-4 fold increase in serum GH. Here we studied developmental changes in skeletal acquisition in a state of serum IGF-1 deficiency. LID mice were followed from 4 to 52 weeks of age. Histomorphometry at 4, 8 and 20 weeks of age in both control and LID mice revealed no changes in bone volume fraction (%BV/TV) and a mild increase in eroded surface at 20 weeks of age (Table 1). Flow cytometry analysis of bone marrow-derived cells revealed a significant and consistent decrease in CD11b/Mac-1+ cells in LID mice, suggesting reductions in osteoclast progenitors in marrow of LID mice. Femoral microCT analyses showed reductions in total area (TtAr), marrow area (MaAr), and cortical area (CtAr) in LID relative to control mice. No differences in matrix mineralization were noted at any age. Morphological differences were apparent at 4 weeks, but were significant at 8 and 16 weeks, indicating that the reduced serum IGF-I affected bone postnatally, at the time when serum IGF-I normally peaks. The reduced serum IGF-I thus had two primary effects on bone; (1) it resulted in slender bones and (2) it impaired structural adaptation necessary to compensate for the increased slenderness (i.e. through reduced marrow expansion and/or increased matrix mineralization). These data suggest that serum IGF-1 is a developmental determinant of bone size and strength, and that compensatory increases in serum GH are insufficient for gaining structural adaptations but may account for the increased resorption at later ages.

Table 1: Histomorphometry of the distal femur from control and LID mice

	4 weeks		8 weeks		20 weeks	
	Control	LID	Control	LID	Control	LID
BV/TV (%)	17.4+/-1.8	16.5+/-1.2	9.0+/-0.6	7.9+/-0.5	6.4+/-0.3	5.6+/-0.4
ES/BS (%)	12.3+/-0.4	10.7+/-1.3	8.9+/-0.5	7.9+/-0.7	12.0+/-0.6	17.2+/-1.2
TbTh (um)	14.0+/-0.9	13.1+/-0.5	13.2+/-0.4	11.7+/-0.3	12.1+/-0.5	10.5+/-0.6
Nob/Tar (/mm^2)	692.9+/-111.1	696.5+/-54.4	416.8+/-14.6	412.7+/-21.2	228.8+/-20.0	246.4+/-23.4
Noc/Tar (/mm2)	74.4+/-10.7	71.8+/-11.8	28.9+/-1.8	24.3+/-2.0	59.6+/-4.7	89.6+/-10.7
TbSp (um)	67.9+/-5.3	66.7+/-4.1	137.1+/-8.1	139.8+/-8.0	178.9+/-10.9	181.2+/-14.2
TbN (/mm)	12.3+/-0.8	12.6+/-0.7	6.8+/-0.3	6.7+/-0.3	5.4+/-0.3	5.4+/-0.4
MAR (um/day)			0.4+/-0.0	0.5+/-0.0	0.1+/-0.0	0.1+/-0.0
BFR (um3/um2/day)			0.024+/-0.00	0.026+/-0.00	0.004+/-0.00	0.005+/-0.00

***Disclosures:* S. Yakar**, *None.*
This study received funding from: NIH.

SA184

Endocrine IGF-1 Maintains Linear Growth in the Total Absence of Tissue IGF-1. Y. Wu*, S. Elis*, H. Sun*, Y. Kawashima*, S. Yakar. Endocrine, Mount Sinai School of Medicine, New York, NY, USA.

Insulin-like growth factor-I (IGF-I) plays a fundamental role in growth and development, and acts in an endocrine and autocrine/paracrine fashion. A daunting challenge has been to sort out which mode of IGF-I action (i.e. endocrine or autocrine/paracrine) facilitates skeletal growth. We previously generated the liver-specific IGF-I gene deletion (LID) mouse model that demonstrated a 75% reduction in circulating IGF-I levels and exhibited mild growth abnormalities, suggesting an important role for tissue IGF-1. Here we took a reversed approach and created a mouse model with total IGF-1 gene-deletion (KO), that expresses Hepatic IGF-I Transgene (HIT) under the control of the transthyretin promoter. The KO-HIT mice express solely liver-derived IGF-I, show 2fold increase in serum IGF-I levels and exhibit complete absence of tissue IGF-I. Serum levels of GH and the IGF binding proteins in KO-HIT mice are similar to controls. Furthermore, body weight of KO-HIT mice was similar to controls in both males and females from 3-16 weeks of age. We reveal that in the total absence of tissue IGF-I action, endocrine IGF-I restores femoral linear growth in both males and females. These findings call into question previous concepts about the GH/IGF-1 axis and have major implications for various growth disorders and therapeutic options.

***Disclosures:** **S. Yakar**, None.*
This study received funding from: NIH.

SA185

Induction of Oxidative Stress and Diversion of β-catenin from TCF- to FOXO-mediated Transcription by Glucocorticoids or TNFα in Osteoblastic Cells. M. Almeida, E. Ambrogini, M. Martin-Millan, L. Han, A. Warren*, R. S. Shelton*, L. Plotkin, T. Bellido, C. A. O'Brien, R. L. Jilka, R. S. Weinstein, S. C. Manolagas. Center for Osteoporosis and Metabolic Bone Diseases, University of Arkansas for Medical Sciences and Central Arkansas Veterans Healthcare System, Little Rock, AR, USA.

Oxidative stress induces activation of the FoxO family of transcription factors and diverts β-catenin from Wnt induced TCF/Lef- transcription to FoxO-mediated transcription--thereby decreasing osteoblastogenesis. Prompted by evidence that oxidative stress is also a causal mechanism of the insulin resistance produced by glucocorticoids (GC) and the inflammatory cytokine TNFα alike, we tested the hypothesis that the potent suppressive effects of GC and TNFα on bone formation may be caused, at least in part, by increasing oxidative stress and antagonizing the beneficial effects of Wnt signaling on osteoblast generation and survival. We report that dexamethasone (Dex) (10^{-7} M) or TNFα (10^{-8} M) increased reactive oxygen species levels in osteoblast progenitors (C2C12 cells). Moreover, Dex- or TNFα-induced apoptosis in C2C12, committed osteoblasts (OB6), and osteocytes (MLO-Y4), was abolished by the antioxidant N-acetyl-L-cysteine (NAC), the glutathione peroxidase mimic ebselen, as well as catalase, the enzyme that converts H_2O_2 to water. Consistent with a pro-oxidant effect, Dex or TNFα increased the phosphorylation of the adapter protein $p66^{shc}$--a redox enzyme that generates mitochondrial H_2O_2 to trigger mitochondria swelling and apoptosis. In addition, TNFα or Dex, similar to 100 μM H_2O_2, increased the activity of a FoxO-luciferase reporter construct and the transcription of the pro-apoptotic gene Bim (a known FoxO target). The stimulation of $p66^{shc}$ phosphorylation as well as the activation of FoxO-luc by Dex or TNFα were abolished in cells pre-treated for 1 h with NAC. Moreover, the effect of Dex or TNFα on apoptosis was prevented by a dominant negative FoxO construct. On the other hand, TNFα or Dex suppressed the Wnt3a-induced activation of a TCF-reporter construct, as well as alkaline phosphatase activity. In agreement with the in vitro data, implantation of slow-release pellets containing prednisolone for 28 d in 5-month-old C57BL/6 male mice increased ROS levels in the bone marrow, as well as $p66^{shc}$ phosphorylation and Bim gene expression in bone. All these effects were prevented by administration of NAC. We conclude that activation of FoxO transcription factors by oxidative stress represents a previously unappreciated cell-autonomous mechanism of Wnt/β-catenin antagonism contributing to the adverse effects of aging, GC excess and inflammatory cytokines on bone alike.

***Disclosures:** **M. Almeida**, None.*

SA186

See Friday Plenary number F186.

SA187

Monoclonal Antibody to Transforming Growth Factor β Inhibits Tumor Burden and Osteolysis in a Pre-clinical Model of Bone Metastasis. S. Biswas[1], C. Wilburn*[2], S. A. Munoz*[2], J. A. Sterling[1], S. Lonning*[3], G. R. Mundy[4]. [1]Cancer Biology, Vanderbilt University, Nashville, TN, USA, [2]Vanderbilt Center for Bone Biology, Vanderbilt University, Nashville, TN, USA, [3]Genzyme Corporation, Framingham, MA, USA, [4]Vanderbilt Center for Bone Biology, Vanderbilt University, Nashville, TN, USA.

Breast cancer is the most common cancer and the second leading cause of cancer-related deaths for women in the United States. Breast cancer cells metastasize to bone with high affinity. Transforming growth factor β (TGFβ), one of the most abundant cytokines found in the bone matrix, is secreted in active form at the site of osteolytic bone resorption. By acting on both the bone microenvironment and cancer cells, TGFβ acts as a major driver of the vicious cycle of bone metastasis. Inhibiting TGFβ signaling in breast cancer cells by the use of dominant negative receptor constructs or the use of small molecular inhibitors of the receptor kinase have been shown to reduce tumor burden and bone lesions in preclinical breast cancer metastasis models. However, small molecule kinase inhibitors often show non-specific effects on unrelated kinases, and thus have potential for unwanted side effects. Therefore, we have taken a different approach, by which we block all three isoforms of TGFβ systemically using a pan-TGFβ antibody, 1D11(Genzyme). Using MDA-MB-231 human breast cancer cells in a cardiac injection model, we have investigated the efficacy of 1D11 on the following:
(1) metastatic progression of breast cancer cells in bone and
(2) modulation of bone lesions in tumor bearing mice.
We have demonstrated that, treatment with 1D11(10mg/ Kg, three times a week for two weeks) reduces the number of bone lesions (Control: 7.4, Treated: 2.3, P<.005), area of bone lesions (control: 7535.5 cm2, treated: 865.5 cm2, P<.001). Increases in the bone volume as measured by the ratio of BV/TV (Control: 0.093, Treatment: 0.198, P=.01) using micro-CT analysis of tibia. Mice were treated with either 1D11 or control antibody (13C4) i.p. three times every week for two weeks. Treatment was initiated two weeks after tumor inoculation. These results show that 1D11 effectively reduces tumor burden and bone lesions in this preclinical model of human breast cancer metastasis to bone.

***Disclosures:** **S. Biswas**, None.*

SA188

See Friday Plenary number F188.

SA189

Tissue-Engineering and Co-culture System Based Disc Regeneration Mesenchymal Stem Cell's Role. M. Nan*[1], S. Moon[2], C. Lee*[1], J. Lee*[1], E. Kwon*[1], H. Kim*[3], K. Lee*[3], H. Lee*[2], E. Moon*[2], H. Kim*[2], H. Kim*[2], I. Kwon*[1], H. Chun*[4], S. Park[5]. [1]Yonsei University College of Medicine, Seoul, Republic of Korea, [2]Department of Orthopedic Surgery, Yonsei University College of Medicine, Seoul, Republic of Korea, [3]Allograft and Biotechnology, Korea Bone Bank Co.,Ltd, Seoul, Republic of Korea, [4]Kyung Hee University, Seoul, Republic of Korea, [5]Department of Orthopedic Surgery, Korea University, Seoul, Republic of Korea.

Intervertebral disc(IVD) degeneration is caused by loss of water content in nucleus pulposus(NP) which is resulted from proteoglycan and type II collagen reduction. To regenerate IVD, transforming growth factor-beta1(TGF-beta1) and bone morphogenetic protein-2(BMP-2) can be utilized. As a technique of tissue engineering, composite IVD implants are fabricated as novel materials for disc replacement. Mesenchymal stem cells (MSCs) are known to be multipotent in tissue regeneration. Hence, the object of this study was to examine MSC based co-cultured cells in atelocollagen type I scaffold under the influence of TGF-beta1 and BMP-2. NP cells and/or MSCs were cultured at the rate of 0:1, 1:1, 1:0 in type I atelocollagen scaffold. And culture media was added with 5% FBS including TGF-beta1 of 10ng/ml, BMP-2 of 100ng/ml respectively. [^{35}S] Sulfur incorporation for proteoglycan synthesis and [^{3}H]-thymidine incorporation for proliferation were measured. RT-PCR was performed to assess the aggrecan, collagen type I and II, osteocalcin mRNA expressions. As result, co-cultures of MSC and NP cells in scaffold showed significant decreases in proteoglycan synthesis compared to those of NP cells only ($p<0.05$). Co-cultures in scaffold with TGF-beta1 or BMP-2 demonstrated increased newly synthesized proteglycan compared to simple co-cultures ($p<0.05$). NP cell culture in scaffold with TGF-beta1 or BMP-2 showed significant increase in proteoglycan synthesis compared to co-culture. Aggrecan and collagen type II mRNA expression were more pronounced in NP cell culture alone than co-cultures. Adding MSC to NP cells in atelocollagen type I scaffold did not resulted in increased matrix synthesis consequently and chondrogenic phenotype expression and MSC based tissue engineering for disc regeneration seems not to be effective.

***Disclosures:** **S. Moon**, None.*
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SA190

See Friday Plenary number F190.

SA191

Analytical Validation of Diasorin Liaison and Roche Elecsys Methods for the Determination of Osteocalcin. E. Cavalier*, P. Delanaye*, A. Carlisi*, J. Chapelle*. Clinical Chemistry, University Hospital of Liege, University of Liege, Liege, Belgium.

Osteocalcin (OSC) determination is not an easy task. Indeed, "intact" OSC (AA 1-49) is unstable because it is cleaved between AA 43 and 44. This generates an N-mid fragment (AA 1-43), much more stable than intact OSC. The antibodies used in the kits must thus recognize both OSC and N-mid fragment.
The aim of our study was to validate 2 automated methods (Diasorin Liaison (LIA) and Roche Elecsys (ELEC)) designed for the determination of OSC in serum.
Both methods were found to be sensible (limit of detection < 0.50 ng/mL) and reproducible (intra- and inter-assay variation respectively <2 and <5%).
We performed a recovery test according to the NCCLS recommendations. This showed a mean recovery of 98% for LIA and 97% for ELEC. The dilution of 3 sera containing elevated levels of OSC showed a slightly better linearity for ELEC than for LIA (mean recovery: 98 vs. 115%).
When we compared the results obtained with the 2 methods on 50 patients, the Bland-Altman test showed a mean difference of 6.9 ng/mL and a clear tendency for ELEC to give higher results than LIA in patients presenting elevated OSC levels. The results obtained with ELEC were significantly higher (31%; $p<0.0001$) than those obtained with LIA.
We studied the stability of OSC at room temperature (RT), +4°C and -20°C on 10 fresh samples. Our results showed that OSC was stable up to 3 days at RT. At 4°C, a significant ($p<0.05$) but slight increase of maximum 6% was observed after 3 days. At -20°C, we also observed a significant ($p<0.05$) increase of 8.5% after 2 days, but a decrease of 9 and 11% respectively after 15 and 30 days ($p<0.05$).
In non-menopausal women, the reference range published for ELEC is broader than LIA (<31 ng/mL vs. <24 ng/mL respectively) which is consistent with our observations, whereas in post-menopausal ones, LIA's reference range are surprisingly higher than ELEC (<59 ng/mL vs. <46 ng/mL). This discrepant observation may be attributable to the different means used by the 2 societies to establish their reference range. Indeed, Diasorin selected patients with 25-OH vitamin D levels >20 ng/ml and estimated the higher and lower limits on a Gaussian distribution (mean±2 SD). On the other hand, Roche evaluated the reference range in women with no hormonal substitution treatment and took the percentiles 5 and 95 as normal limits.
In conclusion, these 2 techniques perform analytically well but they do not give comparable results. This is due, for one part, to the lack of an International standard against which the societies could calibrate their kits, and for the other part, to the possible difference in the cross reactions between the antibodies and the N-mid fragment.

Disclosures: ***E. Cavalier****, None.*

SA192

High Bone Mass Due to Increased Bone Formation in Mice Lacking the Calcitonin Receptor. J. H. Keller*, A. K. Huebner, P. Catala-Lehnen, T. Schinke, M. Amling. Center for Biomechanics and Skeletal Biology, Department of Trauma, Hand, and Reconstructive Surgery, University Medical Center Hamburg-Eppendorf, Hamburg, Germany.

Although the hormone calcitonin (CT) is well known for its inhibitory effect on osteoclasts, its physiologic function in bone remodelling is still not fully clarified. This is in part due to recent findings showing that the inactivation of CT in mice does not only result in increased bone resorption, but also in increased bone formation. The latter observation was especially surprising, since the calcitonin receptor (CTR) is considered not expressed by osteoblasts. Therefore, in order to analyze, how the inhibitory effect of CT on bone formation can be explained at the cellular level, we took advantage of the Cre-Lox-technology, allowing cell-specific gene deletion in mice. Since the replacement of *CTR* exons 6 and 7 by a neomycin resistance gene has been described to result in lethality of homozygous embryos, but in increased bone formation of heterozygous mice, we inserted loxP sites into introns 5 and 7 of the *CTR* gene, allowing CTR inactivation in cells expressing the *Cre-recombinase*. As a control, mice carrying two "floxed" alleles were first crossed with transgenic mice expressing *Cre* in all cell types, including germ cells, thereby leading to the generation of *CTR+/-* mice with a Cre-independent heterozygous deletion of exons 6 and 7. Surprisingly, when we mated these mice, we obtained living *CTR-/-* offspring, displaying no obvious abnormalities. To verify the success of the approach, we first confirmed the absence of functional CTR in these mice by radioligand binding assays and immunohistochemistry. Thereafter, we embarked on a complete skeletal analysis using static and dynamic histomorphometry. At the age of 3 months we already observed a significantly increased trabecular bone volume in the *CTR-/-* mice, accompanied by an elevated bone formation rate. The same was observed in 6 months old mice, where the differences to wildtype littermates were even more pronounced (BV/TV: 23.0 ± 3.0 % vs. 12.5 ± 0.6 %, BFR/BS: 230 ± 31 $\mu m^3/\mu m^2/y$ vs. 101 ± 6 $\mu m^3/\mu m^2/y$). At the age of 12 months, the bone formation rate was still two-fold higher in the *CTR-/-* mice, but this was accompanied by cortical porosity due to increased bone resorption. Thus, since the observed phenotype resembles the one previously described for mice lacking CT, it appears that the control of bone formation is the major function of the CTR, at least in mice. In addition, having established mice with a "floxed" *CTR* allele should allow answering the question, whether the effect of CT on osteoblasts is mediated indirectly through *CTR* expression in another cell type.

Disclosures: ***J.H. Keller****, None.*

SA193

Subcutaneous Administration Of Salmon Calcitonin: Bone Protective Effect In Adjuvant Arthritis Prevention Model In Rats. J. A. Gasser[1], P. Ingold*[1], A. Venturiere*[1], B. Jost*[2], R. Loeffler*[3], J. Dawson*[2], M. Azria[4]. [1]Musculoskeletal Research, Novartis Institutes for BioMedical Research, Basel, Switzerland, [2]Autoimmunity and Transplantation, Novartis Institutes for BioMedical Research, Basel, Switzerland, [3]Technical Research & Development, Novartis Pharma AG, Basel, Switzerland, [4]Calcitonin Biology & Safety, Novartis Pharma AG, Basel, Switzerland.

Skeletally mature Wistar rats were injected intradermally with a vehicle or 0.1ml of a suspension/rat containing 6mgM Tuberculosis antigen H37 RA (Difco) to induce inflammatory arthritis. Starting from day 0, rats were treated either with daily s.c injections of placebo, salmon calcitonin (sCT) at a dose of 1, 3.2 or 10µg/kg/day, or the reference compound dexamethasone given daily orally by gavage at a dose of 0.1mg/kg/day. Treatment was continued for the entire duration of the study which was 3 weeks. TRAP5b-activity in plasma and bone parameters were measured at 0, 2 and 3 weeks in the proximal tibia metaphysis by pQCT (XCT2000, Stratec Medizintechnik, Pforzheim, Germany) and cancellous microarchitecture by in vivo µCT (vivaCT40, SCANCO Medical, Bruettisellen, Switzerland).
In vehicle treated animals, inflammatory arthritis lead to rapid an continuous decrease in cancellous bone mineral density (Cn.BMD) of 12% at 3 weeks and a corresponding decrease in trabecular bone volume, resulting mostly from the thinning of trabecular elements. Cortical thickness (CtTh) decreased by 14% as a result of endocortical bone resorption while no significant changes in periosteal perimeter was observed. Dexamethasone administration fully prevented joint swelling and all bone changes resulting from inflammatory arthritis. Similarly, calcitonin dose dependently reduced bone loss in both, the cancellous and cortical compartment with the highest dose of 10 µg/kg/day offering full protection. Significant effects were already seen at the lowest dose of 1µg/kg/day sCT. Histomorphometric assessment of TRAP positive cells in the ankle joint confirmed the potent dose-dependent anti-osteoclastic effect sCT, which was in line with indications from plasma TRAP5b measurements. Inhibitory effects were seen in metaphyseal cancellous bone, subchondral bone in the epiphysis, as well as in the peri-articular area.
Studies should be undertaken to investigate the potential of oral formulations of sCT in animal models of inflammatory arthritis. Calcitonin is a potent inhibitor of bone degradation in the inflammatory joint and may be an interesting adjuvant therapy to anti-inflammatory drugs (NSAIDs, DMARDs).

Disclosures: ***J.A. Gasser****, Employe of the Novartis Institutes for Biomedical Research 5.*

SA194

Lack of Calcitonin Accentuates Bone Loss During Lactation by Enhanced Osteoclast Formation and Reduced Osteoblast Formation. C. S. Kovacs[1], B. J. Kirby*[1], J. P. Woodrow*[1], R. F. Gagel[2], N. A. Sims[3]. [1]Memorial University, St. John's, NL, Canada, [2]MD Anderson Cancer Center, Houston, TX, USA, [3]St. Vincent's Institute, Melbourne, Australia.

Lactation in mice induces substantial reductions in bone mineral content (BMC, assessed by DXA) that are reversed rapidly after weaning. *Ctcgrp*-null mice, with a global deletion of the gene encoding calcitonin and calcitonin gene-related peptide, suffer a 55% reduction in BMC during lactation (versus 25% in wild type [WT] mice) followed by complete restoration post-weaning. *Ctcgrp*-null mice also demonstrate upregulated mammary gland PTHrP and serum PTH during lactation, which may increase osteoclast-mediated bone resorption during lactation even further above normal. To assess this, we performed histomorphometry in WT and *Ctcgrp*-null mice during lactation and weaning. Pairs of sister WT and *Ctcgrp*-null mice were sacrificed at day 7 of lactation and at weaning (day 21 after parturition) and histomorphometry was carried out on toluidine blue stained undecalcified sections of vertebrae and tibiae.
During lactation, both WT and *Ctcgrp*-null mice demonstrate reduced trabecular thickness and increased trabecular separation, compared to non-pregnant mice of the same genotype, as well as high osteoclast and osteoid surfaces (OcS/BS and OS/BS). In lactating *Ctcgrp*-null vertebrae and tibiae, OcS/BS was more than double that of WT (vertebrae: 13.9 ± 2.2 vs 4.9 ± 0.6%, $p<0.003$; tibiae: 24.5 ± 4.3 vs 9.6% ± 0.5, $p<0.01$). In contrast, osteoblast parameters were halved in *Ctcgrp*-null vertebrae and tibiae compared to WT (vertebral osteoblast surface [ObS/BS]: 10.7 ± 2.0 vs 20.4 ± 2.9%, $p < 0.03$; OS/BS: 11.7 ± 2.3 vs 21.0 ± 3.0, $p < 0.04$). Large osteocytic lacunae consistent with osteocytic osteolysis were evident in both genotypes.
At weaning, the very high OcS/BS persisted in *Ctcgrp*-null mice. However, between day 7 and 21 ObS/BS quadrupled in *Ctcgrp*-null to 40.0 ± 4.8%, and doubled in WT to 46 ± 2.9%; similar changes were observed in osteoblast number and OS/BS.
Thus, we have confirmed upregulated osteoclast-mediated bone resorption and osteocytic osteolysis during normal lactation. Absence of calcitonin led to a greater increase in OcS/BS, consistent with elevated circulating PTH, but also led to a milder increase in ObS/BS vs WT. The sum of these differences may explain the more substantial bone loss in lactating *Ctcgrp*-null mice. Moreover, *Ctcgrp*-null mice retain the ability to upregulate osteoblast generation to a maximal level at the onset of weaning. This suggests that calcitonin normally dampens the effect of lactation on osteoclasts but not on osteocytic osteolysis, yet enhances the effect of lactation on osteoblasts, and is not required for skeletal recovery after lactation.

Disclosures: ***C.S. Kovacs****, None.*
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SA195

See Friday Plenary number F195.

SA196

Calcitonin Intramuscler Administration for Treating Acute Pain of Osteoporotic Vertebral Commpression Fractures: A Randomized Controlled Trial. T. Nakano*. Orthopaedic Surgery, Tamana Central Hospital, Tamana, Japan.

Calcitonin products have an analgesic effect and vary greatly in dosage form and dose. The most common formulation of calcitonin used in the United States and European countries, etc. is a nasal spray administered on a daily basis, whereas in Japan and other East Asian countries, calcitonin therapy usually consists of intermittent intramuscular administration at relatively low doses such as 20 IU once weekly or 10 IU twice weekly. However, to date only a few clinical investigations regarding the analgesic effect of these calcitonin preparations have been conducted.

This study was designed to assess the usefulness of elcatonin, an eel calcitonin derivative, administered at 20 IU once weekly for pain relief in patients with osteoporosis who had lumbodorsal pain due to a new vertebral compression fracture. The study subjects were 78 hospitalized patients with a diagnosis of a new vertebral fracture based on MRI scan data. They were randomized either to receive elcatonin intramuscularly at 20 IU once weekly or to receive no calcitonin treatment. In these groups, other therapies included the use of a corset from the time of admission to the hospital, and also the use of non-steroidal anti-inflammatory drugs (NSAIDs) was allowed. Each patient was questioned regarding the intensity of pain at the time of getting up from a recumbent position, and the results were assessed using the visual analog scale (VAS). The inquiry to the patient using the VAS was performed by an independent member of the nursing staff who was unaware of the study being conducted.

The results of the study showed a significant reduction of the mean VAS score in patients treated with calcitonin at Week 2 of hospitalization as compared to that determined within 2 days of admission to the hospital, and the tendency remained at Weeks 3 and 4 of hospitalization. In patients with no calcitonin treatment, it was not until Week 4 that a significant decrease in the VAS score was observed; hence improvement of pain was attained earlier in the calcitonin-treated group.

The present data suggest that the therapeutic effect on pain associated with a new vertebral fracture is increased by concomitant use of elcatonin, compared to treatment with a corset and NSAIDs alone. In fact, it has been recognized that calcitonin has a mechanism of action mediated by the central serotonergic neurons, which is different from that of NSAIDs. The results of the study supported this finding.

This study has demonstrated the clinical efficacy of calcitonin intramuscularly administered at 20 IU once weekly for pain associated with osteoporosis-induced vertebral fracture, which has been widely used in the East Asia region.

***Disclosures:** **T. Nakano**, None.*

SA197

The Calcitonin Receptor on Osteoclasts Plays a Physiological Role to Protect Against Hypercalcaemia in Mice. A. G. Turner*[1], F. Tjahyono*[1], W. S. M. Chiu*[1], A. J. Moore*[2], D. M. Findlay*[3], H. A. Morris*[2], J. D. Zajac[1], R. A. Davey[1]. [1]Department of Medicine, University of Melbourne, Austin Health, Heidelberg, VIC, Australia, [2]Hanson Institute, IMVS, Adelaide, Australia, [3]Orthopaedics and Trauma, University of Adelaide, Adelaide, Australia.

We have recently demonstrated using a genetically modified mouse model in which the calcitonin receptor (CTR) is globally deleted (Global CTRKOs), that the CTR plays a physiological role in protecting against induced hypercalcaemia (1). The aim of the present study was to further investigate the mechanism by which the CTR exerts this protective effect. To achieve this aim, we generated mice in which the CTR is deleted specifically within osteoclasts (OCL-CTR KOs) using the Cre/loxP system. At 6 weeks of age, male and female OCL-CTR KO mice were fed a low calcium diet for 2 weeks after which hypercalcaemia was induced by treatment with 0.5 ug 1,25-dihydroxyvitamin D_3 on 2 consecutive mornings. Total serum calcium (Ca) levels were measured immediately prior to, and 45 and 50.5 hours post-first injection. OCL-CTR KO mice display a modest bone phenotype that is currently being examined. Trabecular bone volume/tissue volume (BV/TV) in the femur of female OCL-CTRKOs was decreased by 17% at 6 weeks of age compared to controls ($P<0.05$) while BV/TV in male OCL-CTR KOs was unaffected at 6 weeks of age. Furthermore, no differences were observed in baseline serum Ca and PTH levels between control and OCL-CTR KOs genotypes at baseline, consistent with our hypothesis that the CTR on osteoclasts plays a modest physiological role in regulating bone and calcium homeostasis in the basal state. Peak serum total Ca levels at 50 hours following 1,25-dihydroxyvitamin D_3 induced hypercalcaemia were greater in male OCL-CTR KOs by 31% (0.9mM) ($P<0.05$) compared to controls (Table 1).

Table 1: Total serum Ca levels in OCL-CTR mice versus controls, values are mM+/-SEM.

		0 hrs	45 hrs	50.5 hrs
Male	Control (n=7)	1.79+/-0.06	2.62+/-0.14	2.79+/-0.24
	OCL-CTR (n=5)	1.84+/-0.03	3.23+/-0.20	3.65+/-0.10
Female	Control (n=9)	1.86+/-0.04	2.96+/-0.16	3.18+/-0.16
	OCL-CTR (n=2)	1.78+/-0.01	3.55+/-0.19	3.85+/-0.08

The data for the female OCL-CTR KOs is preliminary and experiments ongoing. We are currently investigating the mechanism for this increased hypercalcaemic response by measuring bone formation markers, osteoclast number and X-laps, as well as the renal Ca handling. In conclusion, we have demonstrated that the biological role of the CTR to protect against induced hypercalcemia in mice is primarily mediated via its action on osteoclasts.

(1) Davey RA, Turner A et al. The Calcitonin Receptor Plays a Physiological Role to Protect Against Hypercalcemia in Mice. JBMR In Press, Accepted 13th March, 2008.

***Disclosures:** **A.G. Turner**, None.*

SA198

Low Serum Osteocalcin Predicts Carotid Plaques and Indicators of the Mtabolic Syndrome. E. Waern*[1], C. Ohlsson[2], J. Kindblom*[2], U. Smith*[3], U. Lerner[4], D. Mellström[1]. [1]Centre for Bone research at the Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden, [2]Centre for Bone Research at the Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden, [3]Dept of Internal Medicine at the Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden, [4]Oral Cell Biology, Umea University, Umea, Sweden.

Introduction: The osteoblast-derived protein osteocalcin has recently been shown to affect adiposity and glucose homeostasis in mice, suggesting that the skeleton via an endocrine mechanism influences energy metabolism (Lee et al. Cell 130:456-469, 2007). The aim of the present study was to investigate the relation between serum osteocalcin and indicators of the metabolic syndrome

Methods and population: 619 randomly selected 70 years old men and women participated in a longitudinal study with examinations at age 70, 76 och 86. BMD was measured in calcaneus with dual photon absorptiometry and osteocalcin was analysed by a double-antibody radioimmuno-assay. Serum was sampled after 10 hours fasting and non-smoking in the morning. Extra- and intracranial circulation was examined by means of duplex sonography and Transcranial Doppler techniques in 142 subjects at age 78.

Results: Osteocalcin correlated inversely to BMD and BMI in both sexes at all ages investigated ($P<0.05$). Osteocalcin increased by 44 percent from 70 to 86 years of age. This increase was related to, but independent of, declining kidney function (cystatin C) and increasing PTH. Serum osteocalcin (adjusted for BMI) correlated inversely to serum insulin $r = -0.11$ ($p<0.01$) and glucose $r = -0.25$ ($p<0.0001$).

A multiple regression model (diabetes excluded) with osteocalcin as dependent variable showed that glucose, (but not insulin), waist circumference and BMD were independent indirect predictors while PTH, cystatin C and ALP were independent direct predictors of osteocalcin ($p <0.001$).

Serum osteocalcin was inversely related to the risk of having bilateral carotoid plaques (OR per SD increase in osteocalcin 0.68 (95% confidence interval 0.47-0.97)). The risk for stroke within 15 years after the age of 70 was increased in men with low serum osteocalcin ($p<0.01$).

Low osteocalcin increased the risk of having high waist circumference (Men > 102 cm, women > 88 cm; OR per SD increase in osteocalcin 0.77 (0.67-0.89). In addition, low osteocalcin predicted one or more parameters of the metabolic syndrome (high waist circumference, BMI >30, high triglycerides, hypertension and diabetes; $p<0.01$).

Conclusion: Low serum osteocalcin predicts high glucose and carotid plaques and indicators of the metabolic syndrome**.**

***Disclosures:** **E. Waern**, None.*

SA199

Oxytocin Directly Regulates Skeletal Homeostasis. R. Tamma*[1], G. Colaianni*[1], L. Zhu*[2], N. Patano*[1], C. Camerino*[1], A. Di.Benedetto*[1], M. Strippoli*[1], G. Greco*[1], G. Montemurro*[1], R. Vergari*[1], L. Mancini*[1], S. Colucci*[1], M. Grano*[1], R. Faccio*[1], J. Li*[2], X. Liu*[2], G. Yang*[2], J. Iqbal*[2], C. Buettner*[2], K. Nishimori*[3], L. Young*[4], I. Bab*[5], L. Sun[2], M. Zaidi[2], A. zallone[1]. [1]Department of Human Anatomy and Histology, University of Bari, Bari, Italy, [2]Mount Sinai Bone Program, Mount Sinai School of Medicine, New York, NY, USA, [3]Tohoku University Graduate School of Agricultural Science, Tohoku, Japan, [4]Center for Behavioral Neuroscience, Department of Psychiatry, Emory University School of Medicine, Atlanta, GA, USA, [5]Hadassah School of Medicine, Jerusalem, Israel.

Oxytocin, a hypothalamic neuropeptide secreted from the posterior pituitary, is indispensible for lactation. Mice lacking either oxytocin or its receptor (Oxtr) are thus unable to lactate, but deliver normally. Loss-of-function studies indicate further roles of oxytocin in the regulation of social behavior, memory, and food intake. Here, we show for the first time that oxytocin directly affects both components of bone remodeling, formation and resorption, and bone mass. Daily subcutaneous injections of oxytocin increase bone mass through a direct effect on osteoblastic bone formation, and also transiently elevate osteoclastogenesis. In contrast, oxytocin and Oxtr deficiency, including haploinsufficiency, cause a dramatic reduction in bone formation resulting in low-remodeling osteopenia. We find that the action of oxytocin is exerted directly on bone cells: preliminary studies reveal no effects of intracerebroventricular oxytocin on osteoclastogenesis or osteoblastogenesis in *ex vivo* bone marrow cell cultures. Oxytocin acts on osteoblasts to stimulate mineralization by enhancing BMP-2 expression, which, in turn, enhances Schnurri-3 causing an up-regulation of the transcription regulators *Osterix* and *ATF-4*. Oxytocin also promotes the production of the osteoclastogenic cytokine RANK-L, which consequently stimulates osteoclastogenesis indirectly. Finally, oxytocin directly stimulates osteoclast formation *via* the NF-κB and MAP kinase pathways, but paradoxically inhibits the

resorptive function of mature osteoclasts through Ca^{2+} signals that stimulate nitric oxide synthase (eNOS) to enhance NO production. We propose that oxytocin has a dominant stimulatory effect on bone formation to ensure optimal bone remodeling and appears indispensible for skeletal maintenance in both sexes. In females, the same hormone that controls parturition and lactation, we believe, facilitates the mobilization of calcium from bone, while protecting overt skeletal loss *via* a powerful bone-forming action. Finally, as oxytocin enhances bone mass, we envisage a therapeutic role for the nanopeptide in human osteoporosis.

Disclosures: ***R. Tamma****, None.*

SA200

Evidence Supporting the Necessity of UDP-N-acetyl-alpha-D-galactosamine-polypeptide N-acetylgalactosaminyl-transferase 3 (GalNac-T3) in the Processing of Fibroblast Growth Factor 23 (FGF23) in Humans. R. I. Gafni, N. Bhattacharyya*, J. S. Brahim*, C. E. Dumitrescu*, T. A. Theman*, M. H. Kelly*, A. A. Molinolo*, M. T. Collins. NIDCR, National Institutes of Health, Bethesda, MD, USA.

FGF23, a protein produced primarily in bone, regulates phosphate and vitamin D homeostasis by enhancing phosphate excretion and suppressing 25-hydroxyvitamin D-1-alpha-hydoxylase in the kidney. This molecule circulates in intact and C-terminus forms, with most of the physiologic activities attributed to the intact molecule. Based on in vitro and animal studies, and observations in humans, the following model has evolved: 1) secretion of intact FGF23 requires O-linked glycosylation by GalNac-T3 and 2) without glycosylation, FGF23 is degraded intracellularly to the inactive C-terminus molecule by widely expressed subtilisin-like proprotein convertase(s) (PCSKs). Genetic conditions associated with increased intact FGF23 result in hypophosphatemic rickets/osteomalacia. Rarely, mesenchymal tumors produce excess FGF23 to cause tumor-induced osteomalacia (TIO). Conversely, mutations which decrease FGF23 or GalNac-T3 expression cause hyperphosphatemic tumoral calcinosis. However, direct evidence supporting the model that GalNac-T3 is present in tissues that produce both FGF23 and PCSKs in humans is lacking. We studied 8 tumors of various cell types (3 fresh frozen, 5 paraffin-embedded) from patients with TIO for the co-production of FGF23, GalNac-T3, and PCSKs. Immunohistochemistry staining was positive for both FGF23 (Immunotopics, San Clemente, CA) and GalNac-T3 (CellMab, Sweden); immunoflourescence demonstrated that FGF23 and GalNac-T3 were frequently co-expressed within the same cell. Expression of mRNA encoding FGF23, GalNac-T3 and several subtilisin-like proprotein convertases was detected by quantitative RT-PCR. Taken together, these findings suggest that the machinery necessary for post-translational modification of FGF23 is contained within these secretory tumors. The presence of GalNac-T3 in these physiologically relevant FGF23-producing tissues, and its co-expression with cells producing FGF23, support the hypothesis that GalNac-T3 is necessary for the processing of intact FGF23.

Disclosures: ***R.I. Gafni****, None.*

SA201

High Expression of the Calcium/Phosphate-Regulating Hormone Stanniocalcin 2 Is Associated with Renal Calcification in the Klotho Mutant Mice. Y. Takei*, H. Yamamoto, M. Masuda*, M. Fukaya*, T. Sato, Y. Taketani, E. Takeda. Clinical Nutrition, University of Tokushima School of Medicine, Tokushima, Japan.

Stanniocalcin (STC) has been identified firstly from the corpuscles of stannius in bony fish as calcium/phosphate-regulating hormone. Two related mammalian stanniocalcin genes, STC1 and STC2, were found to be expressed in various tissues containing intestine, kidney and bone. Klotho mutant (KL) mice as known the model of precocious aging, have hypercalcemia, hyperphosphatemia and hypervitminosis D and exhibit ectopic calcification of vascular media, gastric wall, alveolar wall and kidney. Importantly, recent studies found that the klotho plays as the regulator of fibroblast growth factor-23 (FGF23) signaling and phosphate/calcium homeostasis. It was also previously reported that renal STC2 gene expression was increased in KL mice. However, the regulatory mechanism of STC2 expression through FGF23/Klotho signaling and the physiological role of STC2 in KL mice remains unclear. In this study, we investigated the regulation of STC2 gene expression and it's localization in kidney of wild-type (WT) and KL mice. At six weeks old, Real-Time PCR analysis revealed that the mRNA levels of STC2 in kidney were significantly upregulated in KL mice compared with WT mice, while renal STC1 mRNA levels were no significant alteration. We next performed immunohistochemical analysis to clarify the localization of STC2 in kidney. The focal expression of STC2 was observed in KL mice but not WT mice. In addition, we analyzed renal calcification by von kossa stain, thus ectopic calcification of glomerulus and small artery in the renal parenchyma were detected in KL mice but not WT mice. Interestingly, high expression of STC2 was colocalized with renal calcification in KL mice. These data suggested that highly STC2 expression might be associated with renal calcification in the KL mice.

Disclosures: ***Y. Takei****, None.*

SA202

See Friday Plenary number F202.

SA203

Fibroblast Growth Factor 23 (FGF-23) in Vitamin D Deficient Older Persons. P. Lips[1], J. E. Dijkstra*[2], N. van Schoor*[3], M. Lomecky*[2], V. Chel*[3], M. Vervloet*[4], H. Dijstelbloem*[2]. [1]Department of Endocrinology, VU University Medical Center, Amsterdam, Netherlands, [2]Department of Clinical Chemistry, VU University Medical Center, Amsterdam, Netherlands, [3]EMGO Institute, VU University Medical Center, Amsterdam, Netherlands, [4]Department of Nephrology, VU University Medical Center, Amsterdam, Netherlands.

The phosphatonin FGF-23 promotes phosphate excretion and suppresses renal 1α-hydroxylase leading to low serum 1,25(OH)2D in patients with hypophosphatemic osteomalacia. FGF-23 accumulates with renal failure. The objective of this study was to investigate whether FGF-23 plays a role in the decreased conversion of 25(OH)D into 1,25(OH)2D in older persons with vitamin D deficiency and decreased renal function. Subjects were 39 nursing home residents participating in a vitamin D supplementation study, mean age (SD) 84 years (6.3). Serum 25(OH)D and 1,25(OH)2D were measured by RIA (Diasorin and IDS). C-terminal FGF-23 was measured by ELISA (Immunotopics, San Clemente, CA) interassay CV < 10%. All data are from baseline. Results were: median (25-75th) 25(OH)D 20 nmol/l (14-34), 1,25(OH)2D 50.5 pmol/l (40.3-77.1) FGF-23 60.4 RU/ml (24.0-66.4) and mean (SD) calcium 2.33 (0.11) mmol/l, phosphate 1.01 mmol/l (0.13), albumin 33.6 g/l (3.1), creatinine 99 µmol/l (18). Significant correlations were observed between serum 25(OH)D and 1,25(OH)2D ($R = 0.59$, $p < 0.01$), serum 25(OH)D and creatinine ($R = 0.42$, $P < 0.01$) and serum 25(OH)D and albumin ($R = 0.35$, $P < 0.05$). There was no relationship between serum FGF-23 and vitamin metabolites, phosphate or creatinine. When serum 25(OH)D was dichotomized in levels < 25 and > 25 nmol/l, there was no difference in FGF-23. The low serum 1,25(OH)2D and strong relationship between serum 25(OH)D and serum 1,25(OH)2D confirms the substrate-dependent synthesis of 1,25(OH)2D in vitamin D deficient older persons. These data do not support a role for FGF-23 in the decreased renal synthesis of 1,25(OH)2D in older persons.

Disclosures: ***P. Lips****, Merck and Co 1, 2; Servier 1; Aventis 3; Procter and Gamble 3.*

SA204

Rapid Detection of Intact FGF-23 in Tumor Tissue from Patients with Oncogenic Osteomalacia. M. Mannstadt[1], C. Lorente*[2], H. Jüppner[1]. [1]Endocrine Unit, Massachusetts General Hospital, Boston, MA, USA, [2]Dept. Oral & Maxillofacial Surgery, Massachusetts General Hospital and Harvard Vanguard Medical Associates, Boston, MA, USA.

Oncogenic osteomalacia (OOM) is a rare tumor-induced disease characterized by hypophosphatemia due to decreased renal threshold of phosphate reabsorption, low 1,25-dihydroxyvitamin D concentrations and osteomalacia. The localization of OOM tumors, which often produce excess amounts of the phosphaturic hormone fibroblast growth factor-23 (FGF-23), can be difficult and confirmation of successful tumor removal may require prolonged post-operative observation until normalization of serum parameters is documented. Here, we report the modification of a commercially available intact FGF-23 assay, which enabled us to rapidly document high FGF-23 content in OOM tumor extracts. The assay takes less than 30 minutes to complete and visual inspection of the test plate is sufficient to distinguish positive from negative samples, therefore allowing fast intra-operative assessment of FGF-23 content in OOM tumor extracts.

Several tumors from patients with proven OOM and control tissue were studied. Simple aqueous extracts from small amounts of tumor tissue were prepared and FGF-23 content was evaluated using a commercially available two-site ELISA (Immutopics) that detects intact FGF-23. The assay was performed according to the manufacturer's recommendations ("standard assay"), which takes about four hours, or with shortened incubation times ("rapid assay"), which takes 30 minutes. Extracts from bone and an ovarian tumor served as controls.

When measured by the standard assay, extracts from OOM tumors, but not from control tissues, were shown to contain large amounts of intact FGF-23 (3,600 to >11,000 pg/ml). The rapid FGF-23 assay unequivocally documented high FGF-23 concentrations in all OOM tumor extracts.

In summary, simple aqueous extracts from small portions of six different OOM-associated tumors revealed very high FGF-23 concentrations as assessed by the standard assay, which were also readily detectable by a modified rapid test that takes less than 30 minutes to complete. Similar to intraoperative PTH assays, this rapid assay could thus potentially be performed in or near the operating room, especially since visual inspection of the test plate was sufficient to detect FGF-23 in all six tumors tested. The assay may furthermore help define, intra-operatively, the disease-free margins of tumors located in areas that are difficult to access surgically.

Disclosures: ***M. Mannstadt****, None.*
This study received funding from: NIH.

SA205

See Friday Plenary number F205.

SA206

FGF23 and FGF2 Share a Common but Also Have Distinct Signaling Pathways for Negative Regulation of Bone Nodule Mineralization in Cultured Osteoblasts. T. Minamizaki[1], Y. Yoshiko[1], S. Suzuki*[1], J. E. Aubin[2], N. Maeda*[1]. [1]Oral Growth & Developmental Biology, Hiroshima University Graduate School of Biomedical Sciences, Hiroshima, Japan, [2]Molecular and Medical Genetics, University of Toronto, Toronto, ON, Canada.

Fibroblast growth factor 23 (FGF23) is primarily expressed in osteoblasts/osteocytes and suppresses renal phosphate reabsorption and vitamin D metabolism via the circulation. FGF23 is also targeted to the parathyroid where it suppresses PTH production. Klotho forms a complex with FGF23-FGF receptor (FGFR), which appears to be necessary for FGF23-specific signaling in both tissues. Recently, we established that adenoviral overexpression of FGF23 in the rat calvaria (RC) cell/organ culture models inhibits osteoblast differentiation and bone formation, suggesting that FGF23 acts on bone in an autocrine/paracrine manner independently of its systemic effect on phosphate homeostasis. Similarly to adenoviral overexpression of FGF23, we found that treatment of RC osteoblasts with recombinant FGF23 (rFGF23) impaired bone nodule mineralization, and recombinant (rKlotho) was required for the rFGF23 effect under serum-deprived conditions. RC osteoblasts express only very low levels of Klotho, but Klotho (the secreted membrane isoform, sKlotho) is present in FCS. Removal of sKlotho from FCS by immunoprecipitation eliminated the rFGF23 effect. Consistent with these data, Klotho was detectable by immunofluorescence in RC osteoblasts grown under serum-deprived conditions supplemented with rFGF23 in combination with rKlotho but not without rKlotho. Moreover, FGF23 and FGFR1 were co-immunoprecipitated by anti-FGFR1 and anti-Klotho respectively, only when RC osteoblasts were co-treated with rFGF23 and rKlotho under serum-deprived conditions. These results suggest that FGF23 may act on osteoblasts when circulating sKlotho is present. Not only co-treatment with rFGF23 and rKlotho, but also treatment with rFGF2 alone suppressed matrix mineralization via FGFR1 and ERK activation, and both FGF23 and FGF2 stimulated early growth response-1 mRNA expression. However, FGF23 but not FGF2 is differentially expressed during bone nodule mineralization and is directly regulated by 1,25-dihydoxyvitamin D_3 ($1,25(OH)_2D_3$)-occupied vitamin D receptor at the transcriptional level. Thus, both the non-canonical FGF23 and the canonical FGF2 may act as local factors in bone at least in part via a common ERK signaling pathway. However, activity of FGF23 but not FGF2 may be regulated by circulating sKlotho and $1,25(OH)_2D_3$, suggesting specificity of regulatory pathways of FGF family members in bone formation and mineralization.

Disclosures: ***T. Minamizaki****, None.*

SA207

See Friday Plenary number F207.

SA208

Significance of O-linked Glycosylation of FGF23 Protein. H. Suzuki*, N. Ito, S. Fukumoto, T. Fujita*. Division of Nephrology & Endocrinology, Department of Medicine, University of Tokyo Hospital, Tokyo, Japan.

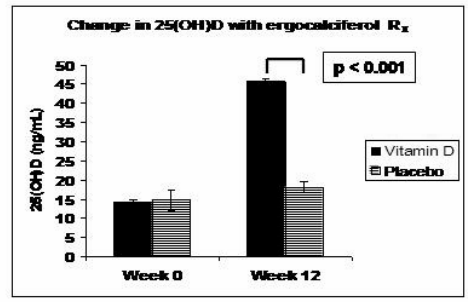
FGF23 is a physiological humoral factor regulating phosphate and vitamin D metabolism. Previous studies indicated that a part of FGF23 protein is proteolytically cleaved between ^{179}Arg and ^{180}Ser into inactive fragments by an enzyme that recognize ^{176}Arg-X-X-^{179}Arg motif. In addition, FGF23 protein has three O-glycan chains at ^{171}Thr, ^{178}Thr and ^{200}Thr. We have shown that these three O-glycans are serially attached to FGF23 protein, first at ^{200}Thr, then at ^{171}Thr and finally at ^{178}Thr. The attachment of the O-glycan chain to ^{178}Thr was shown to prevent the processing of FGF23 protein. However, the significance of O-glycan chains at ^{171}Thr and ^{200}Thr remains unclear. Therefore, we analyzed the mutant FGF23 proteins which lack O-glycan chains at ^{171}Thr and ^{200}Thr in this study. FGF23(Arg176Gln, Arg179Gln) protein is resistant to the processing. Introduction of Thr178Ala mutation into this FGF23(Arg176Gln, Arg179Gln) did not change the presence of FGF23 protein with O-glycan at ^{171}Thr analyzed by Western blotting. In contrast, the FGF23 protein with O-glycan at ^{178}Thr disappeared by the introduction of Thr171Ala mutation. Therefore, O-glycosylation at ^{171}Thr seems to be necessary for the attachment of O-glycan at ^{178}Thr and thereby preventing the processing of full-length FGF23. The introduction of Thr200Ala mutation did not modify FGF23 proteins with O-glycans at ^{171}Thr and ^{178}Thr indicating that the O-glycan at ^{200}Thr does not affect O-glycation at ^{171}Thr and ^{178}Thr. Therefore, we then analyzed biological activity of the mutant FGF23 protein by reporter assay of early growth response-1 (Egr-1) gene in cells expressing Klotho. FGF23 without O-glycan at ^{200}Thr showed less activity compared to that of wild-type FGF23 suggesting that O-glycation at ^{200}Thr enhances the receptor activation by full-length FGF23. Collectively, these results indicate that O-glycans of FGF23 protein contribute to the increased activity of FGF23 either by preventing proteolytic processing or by enhancing the ability of full-length FGF23 to induce intracellular signaling.

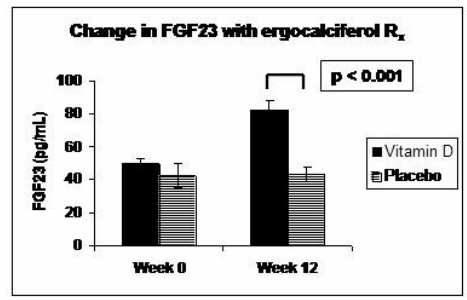
Disclosures: ***H. Suzuki****, None.*

SA209

The Effect of Treating Vitamin D Deficiency on FGF23 Levels in Humans. S. M. Burnett-Bowie, B. Z. Leder, M. P. Henao*, N. T. Mendoza*, J. S. Finkelstein. Endocrine Unit, Massachusetts General Hospital, Boston, MA, USA.

Background: Fibroblast growth factor 23 (FGF23) is a phosphate (PO_4)-regulating hormone that promotes renal PO_4 wasting and suppresses 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) production. Dietary PO_4, serum PO_4, and $1,25(OH)_2D$ appear to regulate FGF23 secretion. Specifically, $1,25(OH)_2D$ stimulates FGF23 production. It is not known, however, if FGF23 is regulated by 25-hydroxyvitamin D (25OHD), the more stable metabolite of vitamin D. Furthermore, the impact of treating vitamin D deficiency on circulating FGF23 is unknown.
Methods: 90 healthy men and women aged 18-45, with 25OHD $\leq$ 20 ng/mL and normal serum calcium (Ca) were randomized to ergocalciferol 50,000 international units or matching placebo (PBO) weekly for 12 weeks. Subjects consumed 1000-1500 mg of Ca daily. FGF23 (*Kainos*, Tokyo, Japan), 25OHD, $1,25(OH)_2D$, serum and urinary PO_4, serum and urinary Ca, and PTH were measured at weeks 0, 4, 8 and 12. The primary endpoint was the change in FGF23 between groups. Data are presented as mean$\pm$SE in the figure and mean$\pm$SD in text.
Results: Subjects were well matched at baseline. 25OHD levels increased from 14±3 ng/mL at baseline to 46±17 ng/mL at week 12 with treatment but were unchanged with PBO (15±4 ng/mL at baseline to 18±10 ng/mL at week 12) ($p<0.001$). Urinary PO_4 excretion increased from 11±5 % at baseline to 13±6 % at week 12 ($p=0.04$) with treatment but was unchanged with PBO. Serum PO_4 and Ca, however, did not change with the intervention. Serum FGF23 increased significantly with treatment from 49±14 pg/mL at baseline to 83±35 pg/mL at week 12 but was unchanged with PBO (43±33 pg/mL at baseline to 43±26 pg/mL at week 12) ($p<0.001$). $1,25(OH)_2D$, PTH and urinary Ca will be presented.
Conclusions: Correction of vitamin D deficiency with high dose ergocalciferol significantly increases circulating serum FGF23, in the absence of any change in serum PO_4. The observed increase in FGF23 is associated with an increase in urinary PO_4 excretion. While dietary PO_4 is generally well absorbed, the observed increase in urinary PO_4 excretion may be a result of increased dietary PO_4 absorption from the correction of vitamin D deficiency. Moreover, these data suggest that 25OHD, or one of its metabolites, has a direct stimulatory effect on circulating FGF23 that is independent of serum PO_4. Future measurement of $1,25(OH)_2D$ levels will help differentiate the effects of 25OHD from $1,25(OH)_2D$ on circulating FGF23.





Disclosures: ***S.M. Burnett-Bowie****, None.*
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SA210

See Friday Plenary number F210.

SA211

Regulation of Renal Klotho: The Importance of ERα. O. K. Oz[1], A. Hajibeigi*[1], K. Korach*[2], P. Chambon*[3], J. Zerwekh[4]. [1]Radiology, UT Southwestern Medical Center at Dallas, Dallas, TX, USA, [2]Environmental Disease Medicine Program, NIEHS/NIH, Research Triangle Park, NC, USA, [3]Physiological Genetic, Inserm, U-596 / CNRS, UMR7104 / Universite´ Louis Pasteur, Illkirch, Strasbourg, France, [4]Internal Medicine, UT Southwestern Medical Center at Dallas, Dallas, TX, USA.

The klotho gene was initially identified in mice harboring a mutation that was associated with several ageing phenotypes including shortened life span, sterility, arteriosclerosis, skin atrophy, muscle atrophy, osteoporosis and abnormal calcium re-absorption. The klotho gene encodes two forms of protein, a type I single transmembrane 130Kda glycoprotein with β-glycosidase activity and a splice variant lacking the transmembrane domain. Full-length klotho is predominantly expressed in tissues involved in calcium homeostasis, such as kidney, parathyroid glands and choroids plexus in the brain while the secreted form is found in the blood and cerebrospinal fluid. In a recently published study, we showed that the expression of klotho in the kidney of aromatase deficient (ArKO) mice was up-regulated both at the mRNA and protein levels when compared to wild type (WT) animals. Treatment with estradiol lowered klotho levels to WT levels. In the present study we further examined estrogen regulation of renal klotho in both in vitro and in vivo models. In vitro a mouse DCT cell line was grown in FBS or charcoal stripped FBS without or with estrogen as a model of estrogen deficiency or repletion. In vivo we used mutant mice lines (n=3-6 per group) lacking estrogen action through the AF-1 domain of the ERα (ERKOαAF-1-/-) or completely lacking estrogen action through ERα knockout (ERKOα). Finally, we compared the expression of renal klotho between male and female wild-type mice. Klotho expression in kidney homogenates or cell lysates was determined by western blot. The expression of klotho in the DCT cell lysates was increased in cells grown in 10%csFBS compared to FBS. Addition of estrogen to the csFBS decreased klotho levels. In both mutant mouse lines renal klotho expression was significantly higher than wild type. Interestingly, the male WT mice had higher klotho levels than female WT mice. In conclusion our results clearly indicate that in the kidney or a DCT cell line, estrogen down-regulates the expression of klotho. Since the complete and the AF-1 domain ERα knockout are estrogen replete and both show elevated klotho, the data suggest that the AF-1 domain is important for estrogen mediated down regulation of renal klotho.

***Disclosures:* O.K. Oz**, *None.*

SA212

Parathyroidectomy Improves Quality of Life in Hemodialysis Patients with Severe Secondary Hyperparathyroidism. P. G. S. Lacativa*[1], F. M. Franco*[1], C. H. Torres*[1], P. J. M. Patricio Filho*[2], M. D. C. Gonçalves*[3], M. L. F. Farias[1]. [1]Endocrinology, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil, [2]Nephrology, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil, [3]Surgery, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil.

Severe secondary hyperparathyroidism (HPT2) associated with end-stage renal disease (ESRD) leads to several complications that can affect quality of life. Total parathyroidectomy with autologous transplantation (PTX) is an alternative to revert hyperparathyroidism in those patients not submitted to renal transplantation. The aim of this study was to quantify the impact of the severe hyperparathyroidism and of PTX on quality of life. Nineteen patients with severe HPT2, age 42 ± 15 years, were studied before (pre-PTX, mean serum PTH 2434 ± 1821 pg/ml) and one year after parathyroidectomy (post-PTX, mean serum PTH 73 ± 67 pg/ml) and compared with thirty-eight age-matched ESRD patients whose mean PTH was 108 ± 86 pg/ml (control). All patients were maintained on chronic hemodialysis throughout the study. SF-36 questionnaires were used to evaluate quality of life. Pre-PTX patients had significantly lower functional status scores than control patients concerning physical function (30.8 ± 28 vs. 54.6 ± 26.6), physical role limitations (14.5 ± 26.7 vs. 42.8 ± 40), bodily pain (36.3 ± 30.2 vs. 58.7 ± 26.8), vitality (37.6 ± 25.6 vs. 58 ± 21.2) and emotional role limitations (26.3 ± 41 vs. 55.3 ± 44). Refractory bone pain was the only symptom related to the functional status deficits in multivariate regression analysis. Parathyroidectomy had a great impact in symptoms relief, especially bone pain, and improved all functional status deficits scores to the levels found in control patients. We conclude that severe HPT2 causes functional status deficits in daily physical activities, vitality and emotional problems, which are closely related to refractory bone pain. The surgical cure of hyperparathyroidism is effective in reverting pain and therefore has a great and positive impact in health status, allowing patients to recover the expected quality of life of ESRD individuals.

***Disclosures:* M.L.F. Farias**, *None.*

SA213

See Friday Plenary number F213.

SA214

Effects of Intermittent Human Parathyroid Hormone Administration on Bone Union with Hydroxyapatite Blocks at the Site of Cancellous Bone Osteotomy in Ovariectomized Rats. K. Kamo, N. Miyakoshi, Y. Kasukawa, K. Nozaka*, H. Sasaki, Y. Shimada*. Akita University School of Medicine, Akita, Japan.

Although hydroxyapatite (HA) blocks have been widely used for reconstruction of bone defects, bone union with HA blocks is often delayed in osteoporotic patients. Intermittent administration of human parathyroid hormone (h-PHT) accelerates fracture healing by increasing callus formation at cortical bone and enhances bone union at the site of cancellous bone osteotomy in ovariectomized (OVX) rats. However, little is known about the effects of hPTH on bone union with HA blocks. The aim of this study was to evaluate whether h-PTH enhances bone union with HA blocks at the site of cancellous bone osteotomy in OVX rats. Following sham or OVX operation in 7-month-old female Sprague-Dawley rats, complete mid-sagittal osteotomy from the knee joint to the tibial diaphysis was performed. An HA block was then placed into the osteotomy site and fixed with cerclage wiring. Postoperatively, hPTH (100 μg/kg) or vehicle only was administered subcutaneously once a week for 8 weeks (n=3-6 per group). Tibiae were harvested 1 week after last injection and bone histomorphometry at the site of osteotomy with HA blocks was performed to evaluate bone volume (BV/TV), osteoid surface (OS/BS), eroded surface (ES/BS), mineral apposition rate (MAR), bone formation rate (BFR/BS) and percentage of bone union with HA block. PTH treatment significantly increased BV/TV (107%, $p<0.01$), MAR (72%, $p<0.01$) and BFR (115%, $p<0.05$) in OVX rats compared to vehicle-treated controls. PTH did not significantly increase bone union with HA blocks in sham-operated rats. The percentage of bone union with HA blocks in OVX rats was increased (79%) by PTH treatment compared to vehicle-treated controls, but not significantly. Intermittent administration of hPTH stimulated bone formation and increased bone volume at the cancellous osteotomy site placed with HA blocks. Treatment with hPTH tended to stimulate bone union with HA blocks in OVX rats.

***Disclosures:* K. Kamo**, *None.*

SA215

See Friday Plenary number F215.

SA216

PTHrP Is Processed by Skin Keratinocytes and Has Immunomodulation Properties. L. Deftos[1], D. Burton[1], C. Chalberg*[1], K. Smith*[1], S. Tu*[1], R. Dorschner*[2], R. Gallo*[2]. [1]Medicine, Veterans Administration San Diego Healthcare System and University of California, San Diego, CA, USA, [2]Dermatology, Veterans Administration San Diego Healthcare System and University of California, San Diego, CA, USA.

Our recent appreciation of the immunomodulatory effects of PTHrP in keratinocytes sheds new light on the biology of this polypeptide. It is our overall hypothesis that the robust expression of PTHrP in keratinocytes is accompanied by processing of its three polypeptide isoforms into tissue-specific peptides with distinct biological effects that regulate the innate immune system.

In this study, we used an immortalized human keratinocyte cell line (HaCaT) to investigate the processing of PTHrP. Through size exclusion chromatography, westerns, and the use of multiple PTHrP antibodies, we showed the immunochemical and size heterogeneity of PTHrP in HaCaT keratinocytes, providing evidence for its processing in this cell. To further characterize the processed forms, we immunoextracted HaCaT conditioned media with N-terminal PTHrP antibody (1A5) coated beads followed by MALDI-TOF of the eluted fractions. We demonstrated several novel keratinocyte PTHrP species, consistent in masses with human PTHrP 3-30, 3-35, 9-39, and 10-36. We previously showed the biological effects of PTHrP in skin include upregulation of the antimicrobial peptide, cathelicidin, made by keratinocytes. To determine if PTHrP can act directly as an antimicrobial, we evaluated synthetic PTHrP peptides for direct antimicrobial effects on *Staphylococcus aureus* growth. While each of the peptides demonstrated some activity, PTHrP140-173 was the most potent and even more potent than the cathelicidin peptide, LL-37.

In summary, these data identify a novel role for keratinocyte-derived PTHrP in the immune defense of skin. The biological studies of PTHrP peptides and the known interactions of PTHrP with vitamin D indicate that PTHrP has a role in the innate defense system of skin against microbes and other invading agents. Our overall aim is to elucidate the molecular products and pathways of PTHrP processing and to identify the biological effects of these cellular mechanisms in keratinocyte immune function in vitro and in vivo.

***Disclosures:* D. Burton**, *None.*
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SA217

See Friday Plenary number F217.

SA218

At Similar Plasma 25-hydroxyvitamin D Levels, Fat Mass Influence PTH Levels in the State of Vitamin D Insufficiency. L. Rejnmark, P. Vestergaard, L. Mosekilde. Dept. of Endocrinology and Metabolism C, Aarhus University Hospital, Aarhus, Denmark.

A relationship may exist between bone and fat metabolism. Adiposity is associated with increased PTH levels, which have been attributed to secondary hyperparathyroidism due to low plasma 25-hydroxyvitamin D (25OHD) levels. In order to further characterise this relationship, we studied PTH levels stratified by vitamin D status and body mass index (BMI).
In a cross-sectional design, we studied 1097 recent postmenopausal women recruited from the local background population. Subjects were divided into tertiles of 25OHD levels and within each vitamin D tertile we studied whether PTH levels differ according to tertiles of BMI. Results were adjusted for age, daily calcium intake, and smoking. We also determined body composition by DXA.
Within each 25OHD tertile, mean 25OHD levels did not differ according to BMI tertiles. PTH decreased with increased 25OHD levels. In the lowest vitamin D tertile (25OHD < 47nmol/l) PTH levels were significantly higher (4.3 pmol/l) than in the mid (4.0 pmol/l) and in the highest (3.7 pmol/l) 25OHD tertile. Within each vitamin D tertile, PTH levels increased significantly with increased BMI i.e., in the lowest 25OHD tertile those in the highest BMI tertile (>26kg/m^2) had significantly higher PTH levels (4.3 pmol/l) than those with a BMI in the mid (4.1 pmol/l), or in the lowest (3.9 pmol/l) BMI tertile. Similarly, in the mid 25OHD tertile (25OHD 47-73nmol/l) PTH levels were 4.4, 3.9, and 3.7 pmol/l in the highest, mid and lowest BMI tertile, respectively. A similar relationship existed in the highest 25OHD tertile. However, after adjustments the significant relationship only remained within the lowest and mid 25OHD tertile, whereas BMI did not affect PTH levels in subjects with 25OHD levels above 73 nmol/l. Performing the analyses with tertiles of total fat mass instead of BMI revealed similar results, whereas lean tissue mass did not affect PTH levels. Accordingly, in vitamin D insufficiency (in this setting at 25OHD levels < 73 nM) PTH levels are - at similar 25OHD levels - influenced by the size of the total fat mass, whereas no such relationship seems to exist in the state of a sufficient vitamin D status. Further studies are needed to determine the mechanism of action by which fat tissue increases PTH levels or whether PTH by itself may increase fat mass.

***Disclosures:* L. Rejnmark**, *None.*

SA219

See Friday Plenary number F219.

SA220

Role of the Transcription Factor SOX4 in the Skeletal Response to Intermittent PTH. D. D. Pierroz[1], L. S. Nissen-Meyer*[2], S. L. Ferrari[1]. [1]Div of Bone Diseases, Geneva University Hospital and Faculty of Medicine, Geneva, Switzerland, [2]The Biotechnology Centre, University of Oslo, Oslo, Norway.

SOX4 is a transcription factor that is also expressed in the embryonic growth plate and in osteoblast-like cells. Homozygous mice die in utero from cardiac failure. We previously reported that heterozygous SOX4+/- mice have decreased BMD, cancellous and cortical microarchitecture and mineral apposition rate (1). Furthermore, bone biopsies from patients with primary hyperparathyroidism show increased SOX4 mRNA expression compared to parathyroidectomized patients, whereas PTH treatment of osteoblastic cells increases SOX4 mRNA. This led us to hypothesize that SOX4 is involved in the bone anabolic response to PTH. For this purpose, 12-week-old female wild type (WT) and Sox4 +/- mice were treated with hPTH 1-34 (40 ug/kg/d) for 6 weeks or vehicle (veh) (n=9-12mice/goup). Bone mineral density and vertebral and femoral microarchitecture were evaluated by DXA and micro-CT, respectively, and bone turnover by serum biochemical markers.
In WT, PTH treatment significantly increased BMD at total body (TB) (+6.5%), spine (Sp) (+2.4%), and midshaft femur (Fem) (+8.6%) compared to veh ($p<0.05$). Similar BMD gains were observed in SOX4+/- mice (TB:+7.3%, Sp:+12.1%, Fem:+8.3%, $p<0.05$). Femur cortical thickness (CTh) and bone area (BA) were also similarly increased by PTH in WT and Sox4+/- (CTh:+7.2% and +7.6%, respectively; BA:+7.3% and +10.9%, respectively, compared to vehicle, $p<0.05$). Moreover, no differences were observed between WT and SOX4+/- mice on vertebral and distal femur trabecular bone volume fraction (BV/TV), connectivity-density, and trabecular thickness (TbTh) in response to PTH. PTH treatment significantly increased osteocalcin levels (+30%, $p<0.006$) in both genotypes, whereas CTX levels decreased similarly in SOX4+/- and WT, independent of PTH.
Thus, haploinsufficient SOX4+/- adult mice have a low bone mass and microarchitecture but responded adequately to intermittent PTH. Taken together with the evidence that PTH upregulates SOX4 expression in vivo and in vitro, these observations suggest that the level of SOX4 expression achieved in these mice was sufficient to maintain PTH activity on osteoblasts.
(1) Nissen-Meyer LS et al. J.Cell Sci. 2007, 120:2785-2795

***Disclosures:* D.D. Pierroz**, *None.*

SA221

See Friday Plenary number F221.

SA222

Dominant Tissue Accumulation of MIBI in Parathyroid Glands in Uremic Rats. Y. Imanishi[1], H. Sano*[1], H. Hasegawa*[2], Y. Funase*[2], H. Kasahara*[2], T. Minamizawa*[2], M. Inaba[1], T. Miki[3], Y. Nishizawa[1]. [1]Metabolism, Endocrinology and Molecular Medicine, Osaka City University Graduate School of Medicine, Osaka, Japan, [2]FUJIFILM RI Pharma Co., LTD., Tiba, Japan, [3]Geriatrics and Neurology, Osaka City University Graduate School of Medicine, Osaka, Japan.

Little is known about the mechanisms of parathyroid imaging by ^{99m}Tc sestamibi or MIBI, although MIBI is one of the most useful image analyses of enlarged parathyroid glands. MIBI accumulation in parathyroid tissue was investigated in 5/6-nephrectomized uremic rats, exhibiting secondary hyperparathyroidism. 5MBq of MIBI was administered to the rats from tail vein, followed by sacrifices of the rats and corrections the tissues such as parathyroid, thyroid, neck muscle tissues and blood in 2 hours. The ^{99m}Tc radioactivity of parathyroid glands of uremic rats was significantly 2.2-fold higher than that of sham-operated rats. The radioactivity in parathyroid glands of uremic rats was almost same as that of sham rats, when compared with the radioactivity per parathyroid gland weight, suggesting that ^{99m}Tc accumulation in parathyroid cells was not affected by uremia and secondary hyperparathyroidism. The radioactive ratio of parathyroid glands to thyroid glands increased 1.2-fold in uremic rats compared to sham rats. These finding suggested that the mechanisms of parathyroid imaging by MIBI in uremia were partly by increased parathyroid gland volume and the enhanced contrast of radioactivity to thyroid glands.

***Disclosures:* Y. Imanishi**, *None.*

SA223

The C-terminal Fragment of Parathyroid Hormone-Related Protein, PTHrP (107-139), Exerts Osteogenic Features in Both Regenerating and Nonregenerating Bone in Mice with Diabetes-Related Osteopenia. D. Lozano*[1], L. F. de Castro*[1], E. Gómez-Barrena*[2], S. Dapia*[3], F. Manzarbeitia*[4], P. Esbrit[1]. [1]Bone and Mineral Metabolism Laboratory, Fundación Jiménez Díaz, Madrid, Spain, [2]Dpt. of Traumatology, Fundación Jiménez Díaz, Madrid, Spain, [3]Trabeculae (R), S.L., Parque Tecnológico de Galicia, Orense, Spain, [4]Pathology Dept., Fundación Jiménez Díaz, Madrid, Spain.

Type 1 diabetes mellitus (DM) is associated with bone loss. Osteoblastic expression of parathyroid hormone-related protein (PTHrP) -an important modulator of osteoblast differentiation- decreases in age-related osteopenia. The role of C-terminal PTHrP -unrelated to PTH- on bone is currently controversial. We here examined the putative osteogenic effects of PTHrP (107-139) in a mouse model of streptozotocin (STZ)-induced diabetes. In STZ-DM and control mice, bone regeneration was induced by marrow ablation in both tibiae. The intact femurs were used as nonregenerating bone controls. Some diabetic mice were treated with PTHrP (107-139) (100 µg/Kg/every other day, s.c.) for one week before and 6 days after marrow ablation, and then sacrificed. One tibia was decalcified and included in paraffin for histological evaluation. MC3T3-E1 cells were grown in differentiation medium (a-MEM; 50 µg/ml ascorbic acid, 10 mM β-glycerolphosphate, 10% FBS), with or without high glucose (HG) (25 mM) (or mannitol, osmotic control), supplemented (or not) with 100 nM PTHrP (107-139). Gene expression was analyzed by real-time PCR after total RNA isolation. STZ-DM mice had weigth loss (15%). Evaluation of femoral bone structure in these mice by µCT demonstrated a 40% decrease in trabecular number and BV/TV and a 20% increase in trabecular SMI, and no cortical changes, compared to those in control mice. This was associated with a decrease (50-80%) in the gene expression of PTHrP and vascular endothelial growth factor (VEGF), and the RANKL/OPG ratio. In the the regenerating tibia, STZ-DM mice showed a dramatic increase in adipocyte number (10-fold over control), and a 30% decrease in osteoblast number and osteoid surface at the metaphysis.This was related to a decrease (20-40%) in the expression of the aforementioned genes, and also in that of runx2, osterix, osteocalcin, and the PTH1 receptor, and an increase in PPAR-γ2. All of these effects were reversed by PTHrP (107-139) treatment at both bone sites. In vitro, either HG- or mannitol-containing medium induced similar changes in gene expression of the aforementioned factors; all which were also reversed by PTHrP (107-139) in the culture medium. In conclusion, PTHrP (107-139) can induce osteogenic effects in a mouse model of STZ-induced diabetes. These in vitro findings further support that this PTHrP fragment has potential anabolic effects in osteoblastic cells.

***Disclosures:* D. Lozano**, *None.*

SA224

See Friday Plenary number F224.

SA225

Dominant-negative GCMB Mutants Causing Autosomal-Dominant Hypoparathyroidism - Protein Expression and Cellular Localization. M. Mannstadt[1], S. R. Pulusani*[1], C. Silve[2], H. Jüppner[1]. [1]Endocrine Unit, Massachusetts General Hospital, Boston, MA, USA, [2]Inserm u561, Hôpital Saint Vincent de Paul, Université Paris 5, UFR Médicale, Paris, France.

Hypoparathyroidism (HP) is characterized by levels of parathyroid hormone (PTH) insufficient to maintain normal serum calcium concentrations. HP occurs as a sporadic disease or as a familial disorder, and may present as a familial isolated form. Autosomal dominant HP (AD-HP) can be caused by heterozygous activating mutations in the calcium-sensing receptor or by heterozygous mutations in the PTH gene that impair intracellular processing. Autosomal recessive forms of HP (AR-HP) can be caused by homozygous mutations in the genes encoding PTH or glial cells missing B (GCMB), a transcription factor specific for the parathyroid gland. Homozygous loss-of-function mutations of GCMB in humans and null mutations in mice lead to AR-HP; heterozygous carriers appear to be healthy.

We recently identified two different heterozygous, single nucleotide deletions in GCMB in genomic DNA of the affected members of two families with AD-HP. Both mutations (mutA and mutB) are located in GCMB exon 5 and both lead to a shift in the open reading frame and replace the putative second transactivation domain located within the carboxyl-terminal region of GCMB with unrelated amino acid sequence. Consistent with the mode of inheritance, mutant proteins exhibited dominant-negative properties in luciferase assays in vitro, while two previously reported GCMB mutations (R47L and G63S) that are the cause of AR-HP did not affect function of wild-type (WT) GCMB.

We now generated three GCMB-specific antibodies in rabbits against peptides corresponding to residues 111-130 (N1), 225-245 (C1) and 481-500 (C2). Western blot analysis of WT GCMB expressed in fibroblast DF-1 cells using antibodies N1 and C1 revealed a protein band of the expected size of about 60 kDa. As expected, lysates from cells expressing mutA and mutB showed a protein band that was slightly larger than WT protein. Antibody C2, which is directed against the portion of the protein that is replaced in mutA and mutB, also revealed the expected protein band in lysates from cells transfected with WT, but not from cells transfected with mutA or mutB. We also generated plasmids encoding WT or mutant GCMB tagged with GFP at the amino-terminus. In transiently transfected chicken fibroblast DF-1 cells using these constructs, WT or mutant proteins demonstrated normal nuclear localization.

We conclude that the identified GCMB mutations do not impair protein expression or nuclear localization, yet exert a dominant negative effect on the WT transcription factor. The mutant GCMB proteins and the anti-GCMB antibodies will help in the analysis of GCMB functions in adult parathyroid glands.

***Disclosures: M. Mannstadt**, None.*
This study received funding from: NIH.

SA226

See Friday Plenary number F226.

SA227

A Possible Pathogenic Role of Aberrant Klotho Expression in Primary and Secondary Hyperparathyroidism. T. Krajisnik[1], P. Bjorklund*[2], G. Akerstrom*[2], G. Westin*[2], T. Larsson[1]. [1]Medical Sciences, Institution of Medical Sciences, Uppsala, Sweden, [2]Surgical Sciences, Institution of Surgical Sciences, Uppsala, Sweden.

Klotho was recently shown to directly mediate PTH secretion in parathyroid cells in response to low extracellular calcium. In contrast, Klotho inhibits PTH secretion indirectly through the action of FGF23. Abnormal Klotho expression in parathyroid disorders remains to be determined. Herein, we explored parathyroid Klotho expression in patients with primary (pHPT) and secondary hyperparathyroidism (sHPT). Informed consent and approval by institutional ethical committee were obtained. Surgically removed parathyroid glands from patients with pHPT (n=40) and sHPT (n=25) and 4 normal parathyroid tissue specimens were analyzed for Klotho mRNA and protein levels by quantitative real-time quantitative PCR and immunohistochemistry.

In pHPT, Klotho mRNA levels were significantly decreased (n=23) or undetectable (n=17) compared to normal tissues ($p<0.001$). Reduced Klotho protein expression was confirmed by immunohistochemistry. Importantly, Klotho mRNA levels were significantly and inversely correlated with serum calcium ($r= -0.97$; $p<0.0001$), however, no correlation with serum PTH or adenoma weight was observed. In agreement, calcium, but not PTH, dose-dependently decreased bovine parathyroid Klotho expression *in vitro* ($p<0.01$). We also confirmed decreased expression of the vitamin D receptor (VDR) mRNA levels in pHPT subjects ($p<0.01$).

In sHPT, there was a trend of decreased Klotho mRNA expression in a majority of subjects, although not statistically significant compared to normal controls. Since all sHPT patients were on calcitriol treatment, we explored the effect of $1,25(OH)_2D_3$ on Klotho expression. Importantly, $1,25(OH)_2D_3$ dose-dependently increased Klotho expression in bovine parathyroid cells *in vitro* ($p<0.01$).

In conclusion, serum calcium is a major determinant of parathyroid Klotho expression, which may play a significant role in the pathogenesis of pHPT. Suppressed Klotho and VDR mRNA levels in parathyroid adenomas indicate that both FGF23/Klotho- and vitamin D/VDR-dependent inhibitory mechanisms are perturbed and unable to diminish the uncontrolled PTH secretion. Finally, since Klotho expression is induced by vitamin D treatment, the beneficial effect of vitamin D treatment in chronic kidney disease may, in part, be ascribed to enhanced FGF23/Klotho-mediated reduction of PTH secretion.

***Disclosures: T. Krajisnik**, None.*

SA228

See Friday Plenary number F228.

SA229

Anabolic Parathyroid Hormone Treatment Mobilizes Bone Marrow Stromal Stem Cells and Osteoprogenitors Towards Bone Surfaces: Role for the Epidermal Growth Factor Receptor Pathway. J. Zhu, N. C. Partridge, L. Qin. Physiology and Biophysics, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ, USA.

Intermittent injection of PTH dramatically increases bone mass and is one of the most effective treatments for osteoporosis. However, the detailed mechanisms are still largely unknown. Recently, we found that conditioned medium (CM) from PTH-treated UMR 106-01 osteoblastic cells contains factors which are chemotactic for bone marrow stromal stem cells (BMSSCs). In this assay, BMSSCs were seeded in the upper wells of chemotaxis chambers and the lower wells contained CM. We observed a 2-fold increase in the number of BMSSCs migrating toward the lower wells with PTH-treated CM compared with control CM. PTH itself has no chemotactic effect on BMSSCs. Time course experiments indicated that UMR 106-01 cells released chemoattractants within 30 min of PTH treatment and this was maximal at 4 h. Moreover, PTH stimulates MC3T3-E1 and primary calvarial osteoblastic cells to release factors chemotactic for BMSSCs. The migration of BMSSCs in these experiments is not due to random movement (chemokinesis) since there was no increase in the number of migrated cells if BMSSCs were in the upper wells in PTH-treated CM and the lower wells contained control CM. Using the protein synthesis inhibitor, cycloheximide, we demonstrated that PTH-treated osteoblastic cells must synthesize new protein(s) to exhibit chemotactic activity. Interestingly, this chemotactic activity was partially abolished by adding an epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor to the chemotactic assay. Previously, we have demonstrated that the expression of amphiregulin, an EGF-like ligand that signals through the EGFR, is rapidly and transiently stimulated by PTH in osteoblastic cells. Activation of the EGFR pathway stimulates the proliferation of BMSSCs and osteoprogenitors but suppresses their differentiation into mature osteoblasts. In vitro assays showed that all EGF-like ligands are chemotactic for BMSSCs. In vivo, we found that 1 h after the last of 12 days of intermittent PTH injections of adult rats the numbers of CFU-F extractable from bone marrow were decreased. Thus, PTH appears to cause the migration of BMSSCs or osteoprogenitors from bone marrow toward bone surfaces lined with osteoblasts and therefore decreases the numbers of CFU-Fs in the flushed bone marrow. Taken together, our results suggest a novel mechanism for PTH's anabolic actions that PTH stimulates bone lining osteoblasts to produce chemotactic factors, including amphiregulin, which mobilize BMSSCs and osteoprogenitors toward bone surfaces for future proliferation and differentiation into mature osteoblasts.

***Disclosures: J. Zhu**, None.*

SA230

Inhibition of the Mevalonate Pathway Rescues the Dexamethasone-induced Suppression of the Mineralization of Osteoblastic Cells. I. Kanazawa, T. Yamaguchi, S. Yano, K. Hayashi*, M. Yamauchi, T. Sugimoto. Internal Medicine 1, Shimane University Faculty of Medicine, Izumo, Japan.

AMP-activated protein kinase (AMPK) is known to be expressed ubiquitously including osteoblasts and modulate the mevalonate pathway by inhibiting HMG-CoA reductase. In this study, to clarify a physiological role of the mevalonate pathway in glucocorticoid-induced osteoporosis (GIO), we investigated the effects of 5-aminoimidazole-4-carboxamide1-β-D-ribonuclepside (AICAR) (an AMPK activator) and fasudil hydrochrolide (FH) [a specific inhibitor of Rho-kinase (ROK), downstream of HMG-CoA reductase] as well as statins (simvastatin and pitavastatin) (HMG-CoA reductase inhibitors) on dexamethasone (DEX)-induced suppression of the mineralization of osteoblastic MC3T3-E1 cells. Either AICAR (up to 0.5 mM) or FH (up to 10^{-4} M) stimulated bone morphogenetic protein-2 (BMP-2) and osteocalcin (OC) mRNA expression by real-time PCR and mineralization by von Kossa and Alizarin red stainings until 21 days in time- and dose-dependent fashions. Simultaneous addition of either 1 mM mevalonate or 5 μM geranyl-geranyl pyrophosphate (GGPP), which also comprise the mevalonate pathway and act in the downstream of HMG-CoA reductase, antagonized BMP-2 and OC mRNA expression as well as mineralization enhanced by AICAR. On the other hand, DEX (10^{-8}M) increased the expression of BMP-2 antagonists, Follistatin and Dan, and inhibited the mineralization of MC3T3-E1 cells. Under DEX treatments, either AICAR (0.1 mM), FH (10^{-5} M), or statins (10^{-6} M) still stimulated BMP-2 mRNA expression of the cells. Moreover, each agent addition reversed the DEX-induced increase in the expression of Follistatin and Dan as well as the DEX-induced suppression of mineralization of the cells. These findings showed that inhibition of the mevalonate pathway by AICAR, FH, or statins rescued the DEX-induced suppression of mineralization of osteoblastic MC3T3-E1 cells via enhancing BMP-2 expression while reducing its antagonists expression. Thus, the mevalonate pathway seems to be involved in the pathogenesis of GIO, and agents that are capable of inhibiting the pathway may be candidate drugs for the treatment of GIO by augmenting BMP-2 action.

***Disclosures: I. Kanazawa**, None.*

SA231

Proteasome Inhibition Counteracts the Negative Effect of Glucocorticoid Treatment on Bone Metabolism by Stimulating Osteoblasts and Inhibiting Osteoclasts in Vitro. K. Søe*, T. L. Andersen*, T. Lund*, T. Plesner*, J. Delaissé. Clinical Cell Biology and Hematology, Vejle Hospital, IRS/CSFU, Southern Denmark University, Vejle, Denmark.

We have studied if simultaneous treatment with a proteasome inhibitor may neutralize the strong side effects glucocorticoids (GC) have on bone metabolism. The proteasome inhibitor bortezomib (Bz) is used to treat multiple myeloma, and it is reported to have beneficial effects on the bone resorption/formation balance and to partly rescue ovariectomy-induced bone loss in mice. We show here in an in vitro study that combining proteasome inhibition with GC treatment overcomes the inhibitory effect of prednisolone on osteoblasts, pre-osteoblasts and human mesenchymal stem cells (hMSC), and also blocks the stimulatory effect of GC on osteoclasts.
The following osteoblast/stem cell models were used: murine calvarial osteoblast cell line (MC3T3), hMSC (Tert4) and human adipose derived stem cells (hADSC). Pre-osteoblasts were defined as hMSC or hADSC differentiated in vitro with cytokines. Endpoints were metabolic activity, alkaline phosphatase activity and Q-PCR. Osteoclasts were differentiated from CD14+ cells derived from human blood donations by culturing them with MCSF and RANKL. Endpoints were metabolic, TRACP and resorptive activity. The experimental setup was adapted to the pharmacodynamics of both prednisolone and bortezomib. We used prednisolone concentrations equivalent to a daily dose of approximately 40 to 125 mg for 24 to 72 hrs, and Bz as a 3 hrs pulse corresponding to the levels reached in patients treated with 1 to 1.3 mg/m^2.
The in vitro prednisolone treatment proved toxic against osteoblasts but not against pre-osteoblasts and stem cells. However, pre-treating osteoblasts with Bz protected them from toxicity. Furthermore, Bz pre-treatment of pre-osteoblasts and osteoblasts enhanced significantly the expression of key genes such as osteocalcin, osteopontin, collagen type 1 and OPG, compared to treatment with GC alone. In osteoblasts, combined treatment also enhanced Vitamin D receptor expression. In addition, hMSC displayed a significant increase in early marker genes such as ALP activity and expression as well as the expression of osteopontin in response to the combination. Finally, prednisolone rendered osteoclast resorption more aggressive but bortezomib could completely prevent this effect. Thus, we reproduce in vitro the detrimental effects of GC on OB and OC activity and show that proteasome inhibition can overcome them. We expect therefore that a possible way to prevent the induction of GC-induced osteoporosis may be through inhibition of the proteasome or the pathways that the proteasome controls.

Disclosures: ***K. Søe****, None.*

SA232

Effect of a Dissociating Glucocorticoid Receptor Modulator on Bone Cells. K. Nelo*[1], E. Kiiskilä*[1], E. Kallio*[1], J. Ilvesaro*[1], H. M. Surcel*[2], J. Risteli[3], M. Nissinen*[1], A. Nilsen*[4], H. A. Järveläinen*[5], T. S. Scanlan*[4], J. Tuukkanen[1]. [1]Anatomy and Cell Biology, University of Oulu, Oulu, Finland, [2]National Public Health Institute, Oulu, Finland, [3]Clinical Chemistry, University of Oulu, Oulu, Finland, [4]Department of Physiology and Pharmacology, Oregon Health and Science University, Portland, OR, USA, [5]Global Safety Assessment, Astra Zeneca R&D, Montreal, QC, Canada.

Corticosteroid therapy is known to induce side effects like bone loss and osteoporosis. Glucocorticoid receptor modulators may have the potential for a more selective anti-inflammatory profile with fewer side effects compared with traditional corticosteroids. To define the effect of a novel non-steroidal selective glucocorticoid receptor modulator (SGRM), we studied osteoblast proliferation, matrix mineralization and osteoclastogenesis.
The anti-inflammatory effect of a arylpyrazole SGRM and dexamethasone was evaluated by analyzing *in vitro* secretion of interleukin-6 (IL-6) cytokine from lipopolysaccharide (LPS) -stimulated murine macrophage-like RAW 264.7 cells and the morphological changes of the RAW cells. The dissociative effect of the SGRM on MC3T3-E1 pre-osteoblast proliferation was studied with a WST-1 assay, mineralization by using Alizarin red S staining, and type I collagen synthesis by measuring procollagen type I N-terminal propeptide. In addition, common osteoclastogenesis markers RANKL and OPG were analyzed from MC3T3-E1 cells with quantitative RT-PCR. Bone resorption was evaluated using mouse bone marrow hematopoietic stem cells.
The anti-inflammatory effect of 10^{-7} M SGRM was 32% and 69% of the effect of dexamethasone in IL-6 secretion and in the cell size respectively. Dexamethasone decreased osteoblast proliferation on days 3, 7, 10 and 14 and mineralization on day 14, compared with a basal control, whereas the SGRM had no effect. The 10^{-7} M SGRM was also less potent in stimulating RANKL mRNA expression and in inhibiting OPG mRNA expression than was dexamethasone in MC3T3-E1 cells (on day 10). An osteoclastogenesis assay didn't detect any differences between these two compounds. However, the effect of 10^{-7} M SGRM on bone resorption was significantly lower when compared with the effect of dexamethasone.
Our findings indicate that a dissociation effect was detected in osteoblast proliferation and matrix mineralization and partially in osteoclast differentiation and bone resorption. The *in vitro* separation of adverse bone effects from anti-inflammatory effects suggests a possibility of developing glucocorticoids with a more selective anti-inflammatory effect.

Disclosures: ***J. Tuukkanen****, None.*
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SA233

See Friday Plenary number F233.

SA234

Normal Intramembranous Fracture Healing in Mice with Transgenic Osteoblast-targeted Disruption of Glucocorticoid Signalling: Analysis by Microcomputed Tomography. A. J. Weber*[1], G. Li[2], R. Kalak*[3], J. Street*[3], C. R. Dunstan[3], F. Buttgereit*[1], H. Zhou[3], M. J. Seibel[3]. [1]Department of Rheumatology and Clinical Immunology, Charité University Medicine, Berlin, Germany, [2]Musculoskeletal Education & Research Unit, Queen's University Belfast, Belfast, United Kingdom, [3]Bone Research Program, ANZAC Research Institute, The University of Sydney, Sydney, Australia.

The role of endogenous glucocorticoids (GC) in osteoblast differentiation and function is not well understood. Using a transgenic (tg) mouse model of osteoblast-targeted disruption of intracellular GC signalling, we examined whether endogenous GC, or the lack thereof, affect intramembranous fracture healing.
Unicortical bone defects (∅ 0.8mm) were created in tg mice (n= 36) and their wild-type (WT) littermates (n=34) using a drill on the anteromedial aspect of the left tibia. Fracture healing was assessed by X-ray (FAXITRON) and micro-CT analysis at 1, 2, and 3 weeks post-fracture. Micro-CT images were analysed for a Region of Interest covering the immediate defect site to measure the volume of new bone and its mean density.
At week 1 post-fracture, micro-CT imaging of the fracture site demonstrated formation of mineralized bone, which increased in volume and density by week 2. At week 3, healing of the defect was nearly completed in all animals.
One week post-fracture, the amount of newly formed bone (BV/TV) was similar in WT (25%) and tg (30%) animals (p=0.28). At week 2, BV/TV was 33% in WT mice as compared to 31% in tg animals (p=0.77). Mean bone densities of the newly formed bone in the WT and tg groups did not differ significantly at week 1 and week 2.
These preliminary results suggest that disrupting endogenous GC signalling in osteoblasts does not affect direct tibia bone healing as assessed by micro-CT. However, it remains to be shown whether glucocorticoid signalling affects other aspects of fracture healing such as collagen synthesis and structure, bone composition or bone turnover and remodelling.

Disclosures: ***H. Zhou****, None.*
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SA235

Expression of 11ß-Hydroxysteroid Dehydrogenase During Murine Osteoclast Formation. M. Yang*[1], J. R. Harrison[2], B. E. Kream[1]. [1]Medicine, University of Connecticut Health Center, Farmington, CT, USA, [2]Craniofacial Sciences, University of Connecticut Health Center, Farmington, CT, USA.

Glucocorticoids exhibit complex regulatory effects on bone remodeling, affecting cells of both the osteoblast and osteoclast lineages. Glucocorticoids have been shown to signal directly in osteoclasts to increase their longevity and suppress their activity. 11ß-hydroxysteroid dehydrogenase (11ß-HSD1) converts the inactive glucocorticoids cortisone and 11-dehydrocorticosterone to their biologically active forms, cortisol and corticosterone, respectively. While 11ß-HSD1 is expressed by many cell types to provide a source of locally-acting glucocorticoids, only one previous study has shown 11ß-HSD1 expression in osteoclasts (human). The goal of the present study was to determine whether murine osteoclast lineage cells express 11ß-HSD1. Bone marrow monocytes (BMMs) were prepared from 7-week-old CD-1 mice and cultured with 30 ng/ml each of M-CSF and RANKL for 4 days to induce osteoclast formation (TRAP-positive cells with >3 nuclei). Freshly isolated BMMs expressed 11ß-HSD1 mRNA as detected by real time quantitative PCR (qRT-PCR) and 11ß-HSD1 protein as assessed by western blotting with a polyclonal rat 11ß-HSD1 antibody (RAH113). 11ß-HSD1 mRNA levels decreased more than 200-fold during osteoclast formation in BMM cultures. To assess whether the reduction of 11ß-HSD1 expression occurred during the expansion of osteoclast precursors, BMMs were treated with 3 and 30 ng/ml M-CSF for up to 4 days. M-CSF caused a time- and dose-dependent reduction of 11ß-HSD1 mRNA, with the greatest reduction after only one day of M-CSF treatment. To assess whether a reduction of 11ß-HSD1 expression occurred during the differentiation of pre-osteoclasts to mature osteoclasts, the monocytic cell line RAW264.7 was treated with or without 30 ng/ml RANKL. 11ß-HSD1 was expressed in vehicle-treated RAW264.7 cells and its expression decreased 2-fold in RANKL-induced cultures containing osteoclasts. To determine whether 11ß-HSD1 was functional, BMMs were treated with M-CSF and RANKL in the presence or absence of corticosterone and its inactive metabolite 11-dehydrocorticosterone for up to 5 days. Both steroids caused a dose and time-dependent suppression of osteoclast formation, indicating that 11ß-HSD1 was active. These data show that BMMs express 11ß-HSD1 as a means of generating locally active glucocorticoids and that this system declines during osteoclast formation in vitro.

Disclosures: ***M. Yang****, None.*
This study received funding from: National Institute of Health.

SA236

Human Osteoblasts Synthesize VEGF In Response to ACTH. H. C. Blair[1], B. B. Yaroslavkiy*[1], L. Guo*[1], L. Liu*[1], M. Zaidi[2], C. M. Isales[3]. [1]Pathology and Cell Biology, University of Pittsburgh, Pittsburgh, PA, USA, [2]Medicine, Mount Sinai, New York, NY, USA, [3]Medicine, Medical College of Georgia, Augusta, GA, USA.

High-dose glucocorticoids cause bone mineral loss and regional osteonecrosis. Osteonecrosis is an important problem in high-turnover bone such as the femoral head and calcaneus. In the adrenal, adrenocorticotropic hormone (ACTH) maintains corticosteroid production via vascular endothelial growth factor (VEGF) production in stromal cells; a consequence of glucocorticoid treatment is reduced systemic ACTH and adrenal cortical involution. Recently, ACTH receptors were discovered in osteoblasts, and are expressed at high levels in trabecular bone. Further, VEGF is required for bone formation. There are major species differences in glucocorticoid response; rodent bone mass increases with glucocorticoids and osteonecrosis does not occur. Thus, we studied VEGF expression in human osteoblasts in vitro. We confirmed the presence of the ACTH receptor in these cells; ACTH receptors were not present in significant amounts in mesenchymal stem cells or in fibroblasts. In early mineralising human osteoblasts, real time RT-PCR showed that VEGF mRNA was present in very low levels in untreated cells. ACTH, 100 ng/ml, induced VEGF mRNA several log orders, reaching 10% of GAPDH control concentration at 3 hours. It then declined to baseline (near zero) at 18 hours. Immune precipitation showed that VEGF protein accumulates in supernatants of osteoblasts treated with ACTH with or without 100 nM dexamethasone. In all media supporting osteoblast differentiation, without added ACTH, low but detectible levels of VEGF were found in culture supernatants. In keeping with the time course of VEGF mRNA expression, withdrawing ACTH for two days reduced VEGF protein to basal levels. It is likely that ACTH is also produced within bone: its precursor, POMC, is a regulated product of macrophages. In keeping, coculture of human monocytes with osteoblasts greatly increased VEGF production in the absence of added ACTH, although twice-higher levels of VEGF occurred in supernatants with both 100 ng/ml ACTH and 100,000 CD14 cells per cm2 cultured with confluent osteoblasts. Monocytes were not a significant source of VEGF. Interestingly, in monocyte-osteoblast cocultures, dexamethasone reduced VEGF nearly to levels of unstimulated osteoblasts alone, suggesting that ACTH (or possibly other VEGF-stimulating products of monocytic cells in peripheral tissues) may be inhibited by glucocorticoids, in addition to inhibiting pituitary ACTH. Our results indicate that bone pathology, including osteonecrosis, secondary to glucocorticoid administration may reflect, in major part, interference with ACTH-dependent VEGF production that promotes osteoblast growth and survival.

***Disclosures:** **H.C. Blair**, None.*

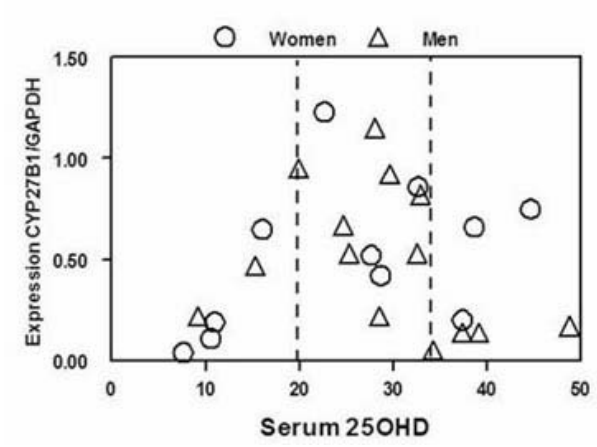
SA237

Expression of 1alpha-hydroxylase (CYP27B1) in Human Marrow Stromal Cells: Correlations with Clinical Status. J. Glowacki[1], S. Zhou[1], M. S. LeBoff[2]. [1]Orthopedic Surgery, Brigham and Women's Hospital, Boston, MA, USA, [2]Endocrinology, Brigham and Women's Hospital, Boston, MA, USA.

1,25-dihydroxyvitamin D [1,25(OH)$_2$D] is important for bone and mineral homeostasis. Recent data show that extrarenal conversion of 25-hydroxyvitamin D (25OHD) to 1,25(OH)$_2$D occurs in several cell types by 25OHD-1alpha hydroxylase (CYP27B1), but little is known about its expression or regulation in bone. We examined 1) whether osteoblast differentiation in human marrow stromal cells (MSCs) responds to 25OHD; 2) whether MSCs express D-related genes; and 3) whether they are regulated. Marrow discarded during orthopedic surgery was obtained from subjects with no comorbidities/ medications affecting bone metabolism; 25OHD (Diasorin RIA), 1,25(OH)$_2$D, and PTH were obtained prior to surgery. Low-density marrow mononuclear cells were isolated by density centrifugation to enrich for undifferentiated cells including a fraction capable of adherence and osteoblastic differentiation. Adherent MSCs were cultured in osteogenic supplements ± 1,25(OH)$_2$D or 25OHD. AlkP activity was measured colorimetrically after 6d. Gene expression was evaluated by semi-quantitative RT-PCR in MSCs obtained from 14 women (W) and 14 men (M). Level of expression was analyzed with respect to clinical parameters.

1,25(OH)$_2$D$_3$ stimulated osteoblast differentiation in 94% samples, with peak stimulation between 1 and 10 nM. Two-thirds of the samples were also stimulated by 25OHD$_3$ (10 nM). This suggests that some marrow may synthesize 1,25(OH)$_2$D$_3$. In the second study, 28% of subjects were vitamin D-deficient (25OHD < 20 ng/mL); 9% had elevated PTH; and 10% had elevated 1,25(OH)$_2$D. There was a wide range of expression of CYP27B1, with no differences between genders or with age (64.8 years ± 11.0). There was lower expression of CYP27B1 in hMSCs from subjects with serum PTH >43 pg/mL (p=0.002) and in subjects with serum 1,25(OH)$_2$D > 50 pg/mL (p=0.0014). There were trends for lower expression of CYP27B1 in MSCs from W and M with serum 25OHD <20 ng/mL, and lower expression in M with serum 25OHD ≥34 ng/mL (Figure). In addition, there was a negative correlation between CYP27A1 and serum 25OHD (p=0.028).

This study indicates correlations between CYP27B1 expression in human marrow stroma and serum 25OHD, 1,25(OH)$_2$D, and PTH. These data suggest that 25OHD and local production of 1,25(OH)$_2$D may play roles in regulation of osteoblast differentiation and function.



***Disclosures:** **J. Glowacki**, None.*
This study received funding from: NIH-NIA

SA238

See Friday Plenary number F238.

SA239

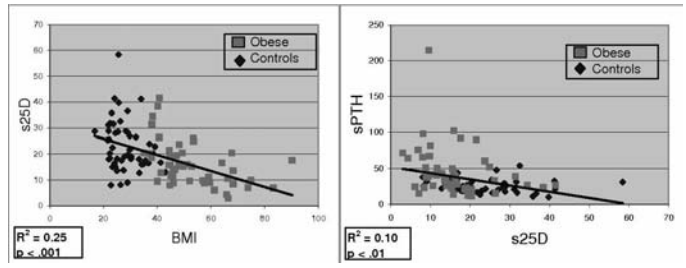
Vitamin D Insufficiency in Obesity. E. R. Grethen*[1], R. V. Considine*[1], R. McClintock[1], C. E. Gupta*[2], R. Jones*[2], B. M. Cacucci*[2], D. Diaz*[2], A. D. Fulford*[1], S. M. Perkins*[1], M. Peacock[1]. [1]Medicine/Endocrinology, Indiana University, Indianapolis, IN, USA, [2]Surgery, St. Vincent's Hospital, Carmel, IN, USA.

Obesity is a major health risk and its incidence in the USA is increasing. There are conflicting reports that serum 25-hydroxyvitamin D (s25D) levels are low in obesity. The aim of this study was to measure s25D in obese and control women and to determine if there was evidence of vitamin D insufficiency as assessed by serum PTH (sPTH).

Fasting blood was obtained from obese white women prior to bariatric surgery and from healthy white women matched for age and season. Height and weight were recorded and s25D and sPTH measured by radioimmunoassay. Data were analyzed by SAS. Mean BMI and sPTH were higher and s25D was lower in obese women (Table).

		Obese women (n = 50)	Control women (n = 50)	P-value
BMI (kg/m^2)	Mean (SD)	52.4 (12.4)	28.1 (5.9)	<0.001
	range	35.0- 90.2	16.7- 43.0	
s25D (ng/ml)	Mean (SD)	16.0 (8.3)	23.3 (9.8)	<0.001
	range	3.0- 41.5	8.0- 58.4	
sPTH (pg/ml)	Mean (SD)	45.2 (33.9)	25.6 (9.1)	<0.001
	range	11.5- 215*	10.2- 54.1	

*Data were analyzed with and without the outlier PTH=215; significance was not affected.
S25D was inversely related to BMI, and sPTH was inversely related to s25D (Figure).



R^2 of s25D vs. BMI was 0.25 (p <0.001); R^2 of sPTH vs. s25D was 0.10 (p<0.01). It is concluded that s25D is low in obesity and that vitamin D insufficiency is present among obese white women.

***Disclosures:** **E.R. Grethen**, None.*

SA240

See Friday Plenary number F240.

SA241

Platelet Vitamin D Receptor Levels Are Reduced in Osteoporotic Patients. P. D'Amelio[1], M. A. Cristofaro*[1], M. Ravazzoli*[1], S. Di Bella*[1], G. P. Pescarmona*[2], G. Isaia[1]. [1]Department of Internal Medicine, University of Torino, Torino, Italy, [2]Department of Biochemistry, Genetic and Biology, University of Torino, Torino, Italy.

Several findings suggest that rapid responses to the action of vitamin D are mediated by a vitamin D receptor (VDR) located in the cytosol. It has been shown that a human megakaryoblastic leukemia cell line expresses a functionally active VDR and that VDR is involved in the control of platelet (PLT) aggregation in the mouse. In the present study, we looked to see whether human PLTs express VDR, and whether its expression is different in osteoporotic as opposed to healthy subjects.
In order support to the analysis of PLT VDR we analyse the VDR expression in megakaryoblasts and megakaryocytes by immunofluorescence (Fig A). We enrolled in the study 77 women with postmenopausal osteoporosis, 33 healthy women of childbearing age, 49 healthy men, and 11 healthy women matched with patients for age and postmenopausal period. Thirty-nine patients had had one femoral fracture occurred after the age of fifty and attributable to primary osteoporosis. Bone mineral density (BMD), markers of bone metabolism and VDR levels by western blot were measured in all the subjects.
We demonstrated that VDR is expressed in the cytoplasm and the nucleus of megakaryoblasts and megakaryocytes, this lends support to the subsequent analysis of PLT VDR. After the menopause, VDR levels increase with respect to the young controls in the healthy women, but not in the osteoporotic ones. Patients with femoral fractures display fewer VDR than those without (Fig B). VDR may thus be supposed to be involved in the control of BMD and the gravity of osteoporosis. There are no marked gender-related differences in VDR expression among the healthy subjects. VDR is directly correlated with lumbar (r=0.4, p=0.04) and femoral (r=0.52, p=0.009) BMD, even after correction for age, time since menopause, BMD, PTH, 25OHvitamin D and BMI. There are no significant correlations between VDR, age and bone metabolism parameters after correction for BMD.
Our data suggest an important role for the VDR in determining bone density, the lower VDR expression in osteoporotic indicate a lower ability to react at circulating vitamin D, and could be the explanation of the increase in the PTH and decrease in the phosphorus levels in patients with respect to controls without difference in circulating vitamin D levels and in the dietary calcium intake.

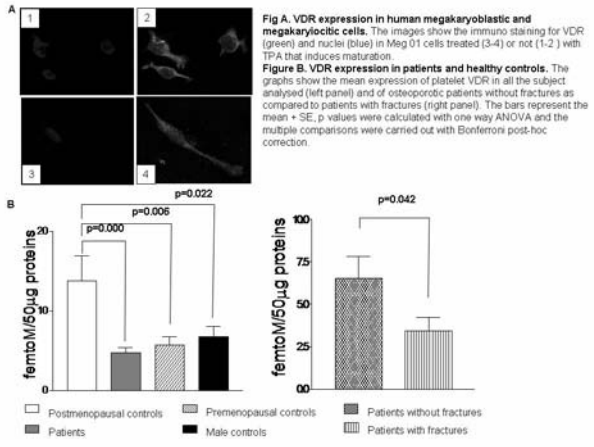


Fig A. VDR expression in human megakaryoblastic and megakaryiocitic cells. The images show the immuno staining for VDR (green) and nuclei (blue) in Meg 01 cells treated (3-4) or not (1-2) with TPA that induces maturation.
Figure B. VDR expression in patients and healthy controls. The graphs show the mean expression of platelet VDR in all the subject analysed (left panel) and of osteoporotic patients without fractures as compared to patients with fractures (right panel). The bars represent the mean + SE, p values were calculated with one way ANOVA and the multiple comparisons were carried out with Bonferroni post-hoc correction.

Disclosures: ***P. D'Amelio****, None.*

SA242

See Friday Plenary number F242.

SA243

Involvement of Caveolae in 1α,25(OH)$_2$D$_3$-dependent Activation of MAPKs in Skeletal Muscle Cells. C. Buitrago*, R. L. Boland. Dept. Biologia, Bioquimica & Farmacia, Universidad Nacional del Sur, Bahia Blanca, Argentina.

1α,25(OH)$_2$D$_3$ is a steroid hormone that induces non-transcriptional events modulating components of signal transduction pathways in various cell types. The ERK1/2 and p38 members of the MAPK family are signaling pathways which mediate rapid responses to extracellular stimuli. Although there are data indicating that 1α,25(OH)$_2$D$_3$ regulates MAPK cascades in skeletal muscle cells, the role of plasma membrane components in the fast effects of the hormone in this tissue have not been investigated. In the present work, using the skeletal muscle cell line C2C12 as experimental model, we demonstrate by Western blot analysis with phosphospecific antibodies that 1α,25(OH)$_2$D$_3$-dependent ERK1/2 and p38 MAPK phosphorylation are suppressed by disassembling caveolae with methyl-beta cyclodextrin (MβCD). Moreover, c-Src activation by the hormone was abolished by exposure of C2C12 cells to MβCD. One of the main molecules of caveolae expressed in this cellular type is caveolin-1 (cav-1). Therefore, we investigated the relationship between cav-1 and c-Src. Confocal microscopy images of immunocytochemical assays of control C2C12 cells showed that cav-1 and c-Src colocalize at the plasma membrane. Cell treatment with 1α,25(OH)$_2$D$_3$ abolished colocalization of cav-1 and c-Src. These results were corroborated by experiments showing that 1α,25(OH)$_2$D$_3$ blocks coimmunoprecipation of cav-1 and c-Src observed under control conditions. Localization of the vitamin D receptor (VDR) in C2C12 cells was investigated. Congruent with previous results, translocation of the VDR to the plasma membrane occurs in cells treated with 1α,25(OH)$_2$D$_3$. In cells exposed to MβCD the VDR is present only in the nucleus. These data suggest that caveolae are involved in c-Src-dependent MAPK activation by 1α,25(OH)$_2$D$_3$ through the VDR in skeletal muscle cells.

Disclosures: ***R.L. Boland****, None.*

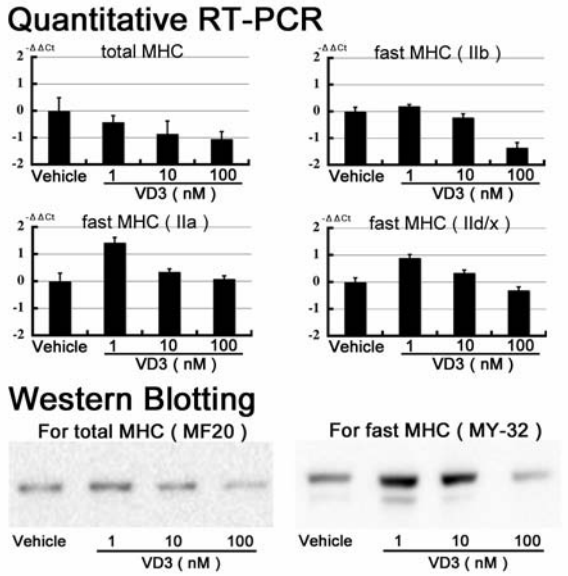
SA244

See Friday Plenary number F244.

SA245

Effect of 1α,25-Dihydroxy Vitamin D3 on Proliferation and Differentiation of C2C12 Myoblast. H. Okuno*, K. N. Kishimoto, M. Hatori*, E. Itoi*. Department of Orthopaedic Surgery, Tohoku University School of Medicine, Sendai, Japan.

Vitamin D plays a major role in the regulation of calcium homeostasis. Recently many reports have demonstrated that 1α,25-dihydroxy vitamin D3 (VD3) supplementation reduced the risk of falls and osteoporosis related fractures of older individuals. An enhancement of the muscle strength and reduction of the body sway are possible mechanisms. However, in vitro effect of VD3 on skeletal muscle has not been well understood. In this study, we examined the effect of VD3 on proliferating, differentiating and differentiated phases of C2C12 myoblasts, mouse skeletal muscle cell line.
C2C12 myoblasts proliferate without fusion and differentiation when grown in 10% fetal bovine serum (FBS). Cell counting and WST-1 cell proliferation assay revealed that supplementation of VD3 under 10% FBS inhibited their proliferation and viability in a dose-dependent manner of VD3. Flow cytometric analysis showed that VD3 increased the percentage of cells in the G0/G1 phase dose-dependently. Cells cultured with VD3 exhibited larger cell size than control under the cytologic observations.
C2C12 cells start fusion and differentiation into myotubes when culture medium is changed to 2% horse serum (HS). VD3 treatment in differentiating phase was defined as starting VD3 supplementation when the medium was changed to HS. VD3 treatment in differentiated phase was defined as starting VD3 supplementation after 8 days culture in HS. VD3 treatment in differentiating phase inhibited the formation of myotube and the expression of total MHC in both mRNA and protein levels. VD3 treatment in differentiated phase did not show significant difference in amount of total MHC using western blot analysis with MF20 antibody (DSHB). However significant larger expression of fast MHC confirmed by Western blot analysis with MY-32 (Sigma) was found in the 1nM of VD3 than in the control.
Our findings indicated that VD3 inhibited proliferation of myoblasts during proliferating and differentiating phases. In the differentiated phase, 1 nM of VD3, a little higher concentration than physiological level, increased the expression of fast MHC. Our result suggested that VD3 might have anabolic effect on skeletal muscle in adequate concentration.



Disclosures: ***H. Okuno****, None.*

SA246

See Friday Plenary number F246.

SA247

The Spectrum of Osteogenesis Imperfecta Mutations in Sweden - A Study of 26 Families. K. M. Lindahl*, C. Rubin*, A. Kindmark, Ö. Ljunggren. Medical Sciences, Uppsala, Sweden.

Background/Aim: Osteogenesis imperfecta (OI) is a heterogeneous disease of connective tissue with an incidence of approximately 1/15 000. More than 90% of OI is caused by a dominant mutation in the genes coding for collagen I, COL1A1 and COL1A2. The cardinal sign of OI is fragile bones with multiple fractures, but the disease can affect many other tissues with symptoms such as blue sclera, excessive bleeding, hyper mobile joints, dentinogenesis imperfecta and deafness. The severity of OI ranges from a mild disease with an average life expectancy to death perinatally. A mild phenotype is often due to a quantitative collagen defect, while patients with more severe forms generally have a mutation leading to a qualitative collagen defect.
Over 800 mutations causing OI have been described in collagen I. As COL1A1 and COL1A2 are large genes, there are still many blank spaces where no mutations have been found and positions where only a few of the possible substitutions have been reported. Approximately 80% of structurally abnormal collagen in OI is the result of a glycine mutation. At present only 11% of possible glycine substitutions in COL1A1 and 10% in COL1A2 have been described.
The mutation spectrum causing OI in Sweden has not been investigated previously. Here the collagen I genes of 26 unrelated patients with OI from all parts of Sweden were studied.
Method: DNA was extracted from whole blood using a commercially available kit. Exons and flanking intron sequences of COL1A1 and COL1A2 were sequenced using standardised sequencing with ABI3130XL using 110 primer pairs.
Results: In 20 of the 26 families a mutation was found: 12 of these were known to cause OI while 8 were not previously reported.
12 mutations were located in COL1A1 and 8 in COL1A2. 11 patients had a glycine substitution, while 7 mutations were insertions, deletions or mutations leading to a premature stop codon. Surprisingly there were 2 non-glycine amino acid substitutions suspected to cause an OI-phenotype and 2 families were carriers of 2 separate mutations. In the latter cases only one of the mutations were of a typical OI-type.
Conclusion: The spectrum of mutations causing OI described in this Swedish cohort are of the expected type with the exception of 2 mutations that cause non-glycine substitutions. In 2 patients 2 separate mutations were found. It is presently unclear if both of these influence the patient's phenotype, but further investigations are planned.

Mutation type and location in cohort of Swedish OI-patients

	COL1A1	COL1A2
Mutations	12	8
Glycine substitutions	4	7
Insertions/deletions/stop codons	7	0
Non-glycine aa substitutions	1	1
> 1 mutation	1	1

***Disclosures:** **K.M. Lindahl**, None.*

SA248

Dynamic Morphological Changes in the Skulls of Mice Mimicking Human Apert Syndrome Resulting From Gain-of-function Mutation of FGFR2 (P253R). X. Du*, X. Zhao*, Z. Chen*, X. Lu*, N. Su*, Q. Sun*, Q. He*, L. Zhao*, L. Chen. Trauma Center, Daping Hospital, Chongqing, China.

The main distinctive craniofacial manifestations of Apert syndrome (AS) include shallow orbits with proptosis, and midface hypoplasia. We have generated a mouse model (Fgfr2+/P253R) mimicking human AS resulting from FGFR2 P253R mutation. Although,in general, this mouse model shows skull phenotypes similar to human AS,the detailed morphometric,especially the dynamic measurement of the skulls of this model was absent. To see if there are precise parallels between phenotypes in Fgfr2+/P253R mouse and human with AS, we meaured, using EDMA approach, the skull morphology of this model and their wild-type controls(WT) at 4W and 8W stages. It was found by FDM (form difference mtrix) analysis that there were significant differences in the overall skull shapes and subsets of anatomic structures between mutants and wt at both 4w and 8w of age. In both stages, compared with WT, the mutants exhibited obviously shortened skulls along the anteroposterior (AP)axis.The anterior portion of the mutant skulls was more severely shortened. In contrast, the mutant skulls were broadened along the mediolateral axis in their frontal regions, while the parietal parts were heightened.The 8w old mutants, however, showed alleviated severity of the shortened anterior portion of the skulls. Of the 351 linear distances constructed from the 27 cranial landmarks, 94% were affected in 4w old mutants, but there were only 76% linear distances showed changes in 8w old mutants. In 8w stage, the mutant skulls had more areas brodened mediolaterally. The percentage of the elongated linear distances in mutant skulls over their littermate WT was increased from 2% in 4w to 5% in 8w.The enlarged distances in 4w old mutants included height of the parietal bones, interorbital distance,maxillary width. In 8w, the width of the frontal bones, breadth of palates and zygomatic bones were also increased.GDM(growth difference matrix) analysis showed that the most significantly increased linear distances were mainly concentrated in the orbit region and anterior portion of the skulls roughly along the AP axis.
Since the skulls of Fgfr2+/P253R developed faster than that in WT mice during the stage between 4w and 8w, it's inidcated that there was a "catch-up" growth in the mutant skulls, especially along the anteroposterior axis, to compensate the retarded development occurred during the early stages.Our data, from cephalometric aspect, further validate the use of the Fgfr2+/P253R mouse model to analyze the pathogenesis of human AS, and develop therapeutic measures for AS.
This study received the "973" program of China (No.2005CB522604),and the NSFC (No.30470947, 30425023).

***Disclosures:** **X. Du**, None.*

SA249

See Friday Plenary number F249.

SA250

Characterisation Through Computer-Assisted Microtomography of the Bone Phenotype of Mice Showing Impaired Gait and Motor Skills. C. Martineau*, L. Brissette*, R. Moreau*. Sciences Biologiques, Université du Québec à Montréal, Montréal, QC, Canada.

Osteoarthritis (OA) designates a group of diseases characterised by stiffness of the joints and ectopic calcification, ultimately leading to erosion of the articular surfaces and consequent movement impairment. Bone architecture alterations for some OA syndromes have been thoroughly documented, such as chondrocalcinosis (CC), craniometaphyseal dysplasia (CMD) and progressive ankylosis, all of which may result from a mutation of the *Ank* gene. This gene encodes a transmembrane channel carrying inorganic pyrophosphate (PPi) to the extracellular environment, preventing excessive calcification. Though CC, CMD and progressive ankylosis show similar symptoms, they are the result of modifications in the N-terminal segment of ANK protein with a gain of function, in the case of CC, and in the C-terminal portion of ANK protein, leading to a loss of function in the case of CMD and progressive ankylosis. Indeed, excessive extracellular PPi results in calcium pyrophosphate dihydrate (CPPD) deposition as seen in CC, while low extracellular PPi leads to hydroxyapatite (HA) formation as observed in the two latter syndromes. In a C57Bl/6 mice colony, 5 individuals of a 6-month-old litter of 9 mice showed abnormal stature and severe locomotor deficiency due, at a first glance, to joint stiffness. The litter was euthanised and the tibiae, femora and lumbar vertebrae were harvested for X-ray microtomography (microCT) analysis. Results confirmed that the articular stiffness was due to excessive ectopic calcification of the toes, knees and spine. Further analysis showed signs of osteoporosis with reduced trabecular tissues, as well as lower bone mineral density (BMD) in tibiae, femora and vertebrae. Alizarin Red S staining of whole mounted legs should discriminate between CPPD and HA formation in the joints, pointing to CC or CMD/progressive ankylosis respectively. Livers harvested and cryopreserved from some individuals will be used for genomic DNA analysis to establish the presence of mutations in the *Ank* gene.

***Disclosures:** **C. Martineau**, None.*
This study received funding from: CIHR.

SA251

Genome-Wide Pleiotropic Associations of Bone Phenotypes: The Framingham SHARe Project. D. Karasik[1], Y. H. Hsu[1], Y. Zhou*[2], L. A. Cupples*[2], D. P. Kiel[1], S. Demissie*[2]. [1]IFAR, Hebrew SeniorLife, Boston, MA, USA, [2]Biostatistics, BU Sch Public Health, Boston, MA, USA.

Genome-wide association studies (GWAS) using high-density genotyping platforms offer an unbiased approach to identify new candidate genes for osteoporosis. There are multiple osteoporosis-related measurements, such as DXA BMD at different skeletal sites. Previously, using a 100K SNP marker set in the Framingham Heart Study (FHS) sample, we (ASBMR 2007) reported that associations with single nucleotide polymorphisms (SNPs) were often shared among multiple bone traits. Here we report results from our FHS SHARe project, with as many as 5 times more SNPs and twice as large sample size.
We used the Affymetrix 500K+50K SNP GeneChip marker set to examine genetic associations with BMD (FN and LS), QUS (BUA and SOS), DXA-derived geometric indices of the hip, and x-ray-derived metacarpal indices, in two cohorts from the FHS. We evaluated 432,159 SNPs with genotypic call rates ≥95%, HWE p≥10-6, and MAF ≥1% in 1554 men and 2073 women (mean age 65 yrs) with individual level call rate ≥97%. Linear mixed effects models (LME) were used to test associations between SNPs and multivariable-adjusted residual trait values. Variance components analysis (SOLAR) was performed to estimate genetic correlations (ρ_g) among these traits. We compared ρ_g among each pair of traits with the proportion of SNPs associated with both of these traits.
We found that at a pre-specified significance threshold α, LS and FN BMD in females share only 2.5% associated SNPs at $\alpha = 0.001$ (5.6% at 0.01 level), despite a high $\rho_g = 0.67$ between them. Similarly, femoral neck length and width, whose $\rho_g = 0.48$ in females, share as much as 1.1% (2.4% at 0.01 level). There are virtually no SNPs that share associations with both BMD and geometric traits (femoral neck and shaft width, neck-shaft angle, and neck length); ρ_g among these geometric traits ranges from -0.20 to +0.48.
In conclusion, GWAS offers a hypothesis-free strategy to identify new associations and may be used to examine pleiotropic relationships between osteoporosis-related traits. Despite some statistical reservations (such as unknown false-positive rate), this strategy may prove helpful to define the best combination of phenotypes to be utilized in genetic studies of pleiotropy in osteoporosis.

***Disclosures:** **D. Karasik**, None.*
This study received funding from: R01-AR050066 and R01-AR/AG 41398.

SA252

Age-Associated Changes in the Material Properties of the G610C (Amish) OI Mouse Model. E. L. H. Daley*[1], D. J. McBride[2], T. E. Uveges*[3], N. V. Kuznetsova*[3], S. Leikin*[3], J. C. Marini[3], S. A. Goldstein[1]. [1]University of Michigan, Ann Arbor, MI, USA, [2]University of Maryland, Baltimore, MD, USA, [3]National Institutes of Health, Bethesda, MD, USA.

The Amish OI knock-in mouse was designed to replicate a COL1A2 G610C mutation associated with OI in an Amish kindred. The mutation has been stably inherited for 6 generations, is found in over 60 individuals, and exhibits a mild, but variable phenotype. The Amish OI mouse expresses mutant type I collagen mRNA and protein. Its mean dermal collagen fibril diameter is reduced compared to WT littermates. Here, we report the first assessment of the G610C mutation on femur geometric and mechanical properties.
Femurs from male 2 month old (WT=9; G610C=9) and 6 month old (WT=9; G610C=6) mice were scanned via µCT to quantify femur morphology and mineralization (GE Medical Systems, London, ON). Cortical regions of interest (ROI) consisted of 3 mm segments of the mid-diaphyses while trabecular ROI were chosen from volumes within the distal femoral metaphyses proximal to the growth plates. Mechanical testing of the femurs was done via 4-point bending conducted with a servo-hydraulic testing system. Predicted material properties were calculated using classical beam theory. One-way ANOVA with *post-hoc* Tukey Test was used to compare group means with a P-value ≤ 0.05 considered significant (SPSS, Chicago, IL).
G610C femurs were shorter, had smaller radii, and had greater volumetric cortical mineral density, but did not differ in cortical thickness compared to age-matched controls. Though cortical morphologies of the mutant genotype were not qualitatively different, voxel-by-voxel image analysis revealed divergent patterns of mineralization in the G610C. Six-month G610C trabecular thickness and mineral density were similar to 6-month controls, but trabecular number was reduced. Two-month G610C yielded and failed at lower loads than the other groups, yet by six-months G610C femurs and controls withstood similar loads. There were no significant differences in yield stress at both ages, but at 6 months failure stress was greater in G610C than control. Though G610C and age-matched controls had similar whole bone stiffness, the G610C mutants had a significantly greater Young's Modulus.
The results demonstrate that G610C has significant phenotypic alterations in bone properties compared to WT. Moreover, G610C femoral material properties appear to alter over time in a way that brings whole-bone strength on par with WT by six months of age, perhaps as part of a mechanism that compensates for the smaller size of the mutant femurs. Interestingly, this compensatory mechanism is remarkably similar to the adaptation observed in Brtl IV mice, suggesting a shared skeletal response for glycine substitutions in either type I collagen chain.

***Disclosures:** **E.L.H. Daley**, None.*

SA253

See Friday Plenary F253

SA254

Characterization of the Enthesopathy of X-linked Hypophosphatemia in the Hyp Mouse. C. M. Macica*[1], G. Liang*[1], L. D. Katz*[2], K. L. Insogna[1], T. O. Carpenter[3]. [1]Internal Medicine, Yale University, New Haven, CT, USA, [2]Diagnostic Radiology, Yale University, New Haven, CT, USA, [3]Pediatrics, Yale University, New Haven, CT, USA.

Despite the characteristic osteomalacia seen in X-linked hypophosphatemia (XLH), paradoxical calcification occurs at tendon and ligament insertion sites, as described over 20 years ago (Polisson, 1985). We confirmed a generalized enthesopathy/osteophytopathy in a clinical survey of over 30 patients affected with XLH; calcaneal spurs and Achilles enthesopathy are often affected earlier than other sites. As previously suggested, enthesopathy does not appear to be determined by phosphate/calcitriol treatment, and is likely intrinsic to the basic disease process. Nevertheless, as no studies to date have examined the progression or pathogenesis underlying mineralization of insertion sites in XLH, we examined several fibrocartilagenous insertion sites in the murine model of XLH, the Hyp (Phex deletion) mouse, including that of the Achilles tendon. Enthesis fibrocartilage (FC) was established by cellular phenotype (small, rounded cells aligned in rows between collagen fibrils) and expression of type II collagen. In contrast to aged-matched control mice (7 mos), the enthesis FC was highly cellular and dramatically thickened, extending well into both the sesamoid and calcaneal FC. The expansion of FC also extended far into the medial process of the calcaneal tuberosity. This was accompanied by a 5-fold increase in expression of type II collagen. In addition, alkaline phosphatase (AP) activity, a general marker of cartilage and bone mineralization, was minimal in the enthesis of control mice, localized only to the enthesis tide-mark. In contrast, a dramatic 20-fold increase in AP activity was observed in Hyp mice and was associated with the expanded FC. It thus appears that the Hyp mouse phenocopies the human syndrome quite precisely and will form the basis our studies characterizing the signaling molecules involved in this process.

***Disclosures:** **C.M. Macica**, None.*
This study received funding from: R01DK62515.

SA255

Possible Central Function of the Calcitonin Receptor on Bone Mass. C. B. Confavreux[1], C. Settembre*[1], F. Oury*[1], R. A. Davey[2], J. D. Zajac[2], G. Karsenty[1]. [1]Genetics and Development Department, Columbia University, New York, NY, USA, [2]Department of Medicine, Austin Health, University of Melbourne, Heidelberg, Victoria, Australia.

Calcitonin, a hormone able to lower serum calcium level, mediates its signal through calcitonin receptor (Calcr), a member of the seven trans-membrane domains G protein coupled receptor family. Since *Calcr* is expressed in osteoclasts, it is generally assumed that calcitonin is a regulator of bone resorption. However, mice lacking one copy of *calcitonin receptor* displayed an increase in bone mass due to an increase in bone formation whereas bone resorption was normal. These in vivo data raise the hypothesis that calcitonin may be a regulator of bone formation, a function that may not be explained by the expression of *Calcr* in osteoclasts. To address this conundrum, we first analyzed *Calcr* expression pattern. Besides osteoclasts, *Calcr* is expressed in neurons of the arcuate nucleus and the paraventricular hypothalamic nucleus (PVH), two hypothalamic nuclei implicated in the control of bone mass. Interestingly, *Calcr* expression in these neurons was markedly stronger than in osteoclasts. This pattern of expression along with the phenotype of *Calcitonin receptor* deficient heterozygous mice raises the prospect that this receptor regulates bone formation through its expression in brain. In an effort to perform a thorough analysis of the function of calcitonin receptor in the different cell types where it is expressed, we used a *Cre-loxP* strategy to generate mice lacking *Calcr* in either all cells ($Calcr_{-/-}$), osteoclasts only through the use of *CathepsinK-Cre* mice ($Calcr_{osc-/-}$) or in neurons through the use of *SynapsinI-Cre* mice ($Calcr_{neurons-/-}$). As expected, $Calcr_{-/-}$ mice displayed an increased bone mass due to an increase in osteoblast number. Remarkably there was no change in bone resorption parameters. Surprisingly, while mice lacking *Calcr* in osteoclasts only lived normally, the brain specific deletion of *Calcr* was lethal, most likely during embryogenesis. We are currently investigating the cause of death in $Calcr_{neurons-/-}$ mice as well as studying the bone phenotype of the $Calcr_{neurons+/-}$ and $Calcr_{osc-/-}$ mice. Results of this analysis will be presented at the meeting.

***Disclosures:** **C.B. Confavreux**, None.*
This study received funding from: Société Francaise de Rhumatologie, Association pour la Recherche sur le Cancer, the Philippe Foundation, Fondation Bettencourt.

SA256

See Friday Plenary number F256.

SA257

An Investigation into the Impact of Osteoarthritic Changes on Bone Mineral Density Measurements in Patients with High Bone Mass. C. L. Gregson*[1], S. Steel*[2], K. Yoshida*[3], D. M. Reid*[3], J. H. Tobias[1]. [1]Bristol University, Bristol, United Kingdom, [2]Royal Infirmary, Hull, United Kingdom, [3]Aberdeen University, Aberdeen, United Kingdom.

High Bone Mass (HBM) is of increasing interest, but so far cases have only been reported sporadically. To identify further HBM cases, we screened 7 Dual Energy X-ray Absorptiometry (DXA) databases in the UK (5 Lunar, 2 Hologic) for T or Z score of ≥ +4 at the lumbar spine or hip. A problem we have met is that since osteoarthritis (OA) can artefactually raise bone mineral density (BMD), and HBM may itself be associated with OA, a method is required to identify HBM cases with or without co-morbid OA, while excluding those with OA alone. To develop this, DXA images from HBM 'cases' identified in this way were graded for OA severity using the Kellgren & Lawrence (KL) score [mild (0-2), moderate (3), severe (4)] and related to BMD results. 562 out of 197,953 scans had a T or Z score of ≥ +4 at the lumbar spine or hip. 77% were women. Mean age 63 years, weight 81.7kg, height 164.6cm. Mean total L1-L4 Z score was 5.09 (3.65-6.55), L1 Z score 3.40 (3.24-3.56), total hip Z score 2.11 (1.97-2.25), KL score 3.36 (3.30-3.46). Subjects with scoliosis (65), ankylosing spondylitis (9), orthopaedic metalwork (8) and other known causes of raised BMD (17) were excluded. In the remaining 463, KL score was compared with BMD using linear regression. Total L1-L4 Z score was strongly correlated with increasing KL score (1.01; 0.54, 1.48; p<0.001); whereas, L1 Z score was not (0.04; -0.53, 0.60; p=0.89). Total hip Z score was negatively correlated with KL score (-0.85; -1.33, -0.38; p<0.001). Total L1-L4, affected most by OA, was thus not appropriate to differentiate HBM from OA; L1 Z score was affected least, reflecting the recognized pattern of progressive OA changes seen in descending sequential lumbar vertebrae. If anything, high hip BMD was negatively associated with spondylosis. Hence a new definition of HBM was developed as a) L1 Z score of ≥ +3.5 plus total hip Z score ≥ +1.2, or b) total hip Z score ≥ +3.5. Using this definition, 179/463 (38.7%) of cases originally identified, after exclusion of other HBM causes, were defined as having HBM ± co-morbid OA, of whom 40.2% had severe and 46.4% moderate OA. In contrast, of the remaining subjects re-classified as non-HBM, 44.8% had severe OA and 45.8% moderate OA (p=0.016). Furthermore, when applying this definition, HBM subjects were much heavier than non-HBM subjects (86.0 [83.4, 88.6] vs 78.2 [76.2, 80.2] kg respectively), whilst height and age were similar, providing further evidence that our revised definition was effective at distinguishing between subjects with or without HBM. In conclusion, L1 and total hip Z scores are useful in identifying HBM (± co-morbid OA), filtering out those with OA alone as an artefactual cause of high BMD.

***Disclosures:** **C.L. Gregson**, None.*
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SA258

PTHR1 Mutations Associated with Ollier Disease Result in Receptor Loss of Function. A. Couvineau*[1], V. Wouters*[2], G. Bertrand*[3], C. Rouyer*[1], B. Gérard*[3], L. Boon*[4], B. Grandchamp*[3], M. Vikkula*[2], C. Silve*[5]. [1]Faculté Bichat, INSERM U773, Paris, France, [2]Université Catholique de Louvain, Laboratory of Human Molecular Genetics, Brussels, Belgium, [3]AP-HP, Hopital Bichat, Service de Biochimie Hormonale et Génétique, Paris, France, [4]Cliniques Universitaires St Luc, Center for Vascular Anomalies, Brussels, Belgium, [5]AP-HP, Hopital Bichat, INSERM U561 et Service de Biochimie Hormonale et Génétique, Paris, France.

The PTHR1 signalling pathway is critical for the regulation of endochondral ossification. Thus, abnormalities in genes belonging to this pathway could potentially participate in the pathogenesis of Ollier disease / Maffucci's syndrome, two developmental disorder defined by the presence of multiple enchondromas. In agreement, a functionally deleterious mutation in PTHR1 (p.R150C) was identified in enchondromas from two of six unrelated patients with enchondromatosis. However, neither the p.R150C mutation (26 tumors) nor any other mutation in the *PTHR1* gene (11 patients) could be identified in another study. To further define the role of PTHR1 signalling pathway in Ollier disease and Maffucci syndrome, we have analysed the coding sequences of four genes (*PTHR1*, *IHH*, *PTHrP* and *GNAS1)* in leucocyte and/or tumor DNA from 57 and 23 patients affected with Ollier disease or Maffucci syndrome respectively. We identified three previously undescribed missense mutations in PTHR1 in patients with Ollier disease at the heterozygous state. Two of these mutations (p.G121E, p.A122T) were present only in enchondromas, and one (p.R255H) in both enchondroma and leukocyte DNA. The assessment of receptor function demonstrated that these three mutations impair PTHR1 function, either by reducing the affinity of the receptor for PTH or reducing receptor expression at the cell surface. These mutations have not be found in DNA from 222 control patients. The PTHR1 mutations identified in Ollier disease (p.G121E, p.A122T, p.R150C) and Blomstrand chondrodysplasia (p.P132L) all lie within the structured core of the PTH-PTH1R extracellular domain complex in the crystal structure described by Pioszak and Xu (PNAS 2008), a location compatible with a functionally deleterious effect. Including our data, PTHR1 functionally deleterious mutations have now been identified in 5/31 enchondromas from Ollier patients. These findings provide further support for the idea that heterozygous mutations in PTHR1 that impair receptor function may participate in the pathogenesis of Ollier disease in some patients.

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SA259

Hypothyroidism and Autism Combined with Pseudohypoparathyroidism in the Absence of Albright's Hereditary Osteodystrophy and *GNAS* Imprinting Changes: A Novel Clinical Syndrome? L. M. Ward[1], T. Pinto*[1], S. Lawrence*[1], M. Lawson*[1], M. Bastepe[2], H. Jüppner[2], F. Rauch[3]. [1]Div. of Endocrinology, Children's Hospital of Eastern Ontario, Ottawa, ON, Canada, [2]Endocrine Unit, Massachusetts General Hospital, Boston, MA, USA, [3]Genetics Unit, Shriners Hospital for Children, Montréal, QC, Canada.

Introduction and Aim: Pseudohypoparathyroidism (PHP) typically arises from defects in *GNAS* (20q13.3), an imprinted gene locus with multiple transcriptional units. Recent studies have shown that the mutations responsible for the PHP-Ib form affect control elements regulating the imprinting of *GNAS*. The purpose of this report was to describe an apparently novel form of PHP-Ib in two brothers with unique clinical features.
Clinical Report: The male propositus presented at 12 years with increasing seizure frequency and autism. Investigations suggested PHP: (Ca^{2+} 0.9 mmol/L, N: 1.1-1.3; PO_4 1.8 mmol/L, N: 1.0-1.7; PTH 54 pmol/L, N: 1.1-6.8). A PTH infusion test (Parathar 3 units/kg) demonstrated only a 6-fold increase in $_u$cAMP (N: >10-fold increase) and a 2.5% reduction in percent tubular reabsorption of phosphate (N: 5-16% reduction). A trans-ilial bone biopsy revealed increased cancellous bone volume (38% above the average) with a 4-fold elevation in bone formation rate. These results confirmed renal resistance to PTH with bone tissue responsiveness. TSH resistance was also suspected: TSH 6.7 mU/L; FT4 <5 pmol/L, TRH stimulation consistent with primary hypothyroidism, negative anti-thyroid antibodies and lack of a goiter. An older brother also manifested autism, PTH resistance and primary hypothyroidism in the absence of positive anti-thyroid antibodies; however, a large goiter was present. Southern blot analyses using gDNA from the propositus and methylation sensitive restriction enzymes showed no evidence of a methylation defect in *GNAS* exon A/B. Analysis of genomic DNA from the affected children and their parents through microsatellite markers located centromeric and telomeric of *GNAS*, and through single nucleotide polymorphisms within the *GNAS* locus showed that the two affected individuals inherited different maternal and paternal alleles.
Conclusions: We describe two brothers with autism, primary hypothyroidism and PHP, where the genetic and epigenetic findings suggest that *GNAS* is not linked to this form of PHP-Ib. This constellation of features may represent a novel clinical syndrome.

***Disclosures: L.M. Ward**, None.*

SA260

See Friday Plenary number F260.

SA261

Genomic Expression Analysis of Rat Chromosome 4 for Skeletal Traits at Femoral Neck. I. Alam[1], Q. Sun[1], L. Liu*[2], D. L. Koller[2], Y. Liu*[3], H. J. Edenberg*[4], M. J. Econs[3], T. Foroud[2], C. H. Turner[1]. [1]Biomedical Engineering, Indiana University School of Medicine, Indianapolis, IN, USA, [2]Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN, USA, [3]Medicine, Indiana University School of Medicine, Indianapolis, IN, USA, [4]Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, IN, USA.

Hip fracture is the most devastating osteoporotic fracture type with significant morbidity and mortality. The primary skeletal determinants of hip fracture risk are bone mineral density, structure and strength. Several studies have demonstrated that there is a substantial genetic component to hip fragility. Thus, finding the genetic basis underlying variation in skeletal traits in femoral neck will facilitate our understanding to prevent and/or treat hip fracture successfully. Genetic linkage studies in humans and animal models identified chromosomal regions linked to hip size and bone mass. Previously, we identified that the region of 4q21-q41 on chromosome (Chr) 4 uniquely harbors multiple femoral neck quantitative trait loci (QTLs) in inbred Fischer 344 (F344) and Lewis (LEW) rats. The purpose of this study is to identify the candidate genes for femoral neck density and structure by correlating gene expression in the proximal femur with the femoral neck phenotypes linked to the QTLs on Chr 4. RNA was extracted from proximal femora of 4-week-old rats from F344, LEW and two other strains (n=4 per strain). Microarray analysis was performed using Affymetrix Rat Genome 230 2.0 arrays. A total of 104 genes in the 4q21-q41 region were differentially expressed ($p<0.05$) among all strains of rats with a false discovery rate (FDR) less than 10%. These 104 genes were then ranked based on the proportion of variation in femoral neck phenotypes in F2 animals homozygous for a particular strain's allele at the Chr 4 QTL explained by the expression level of the gene in that strain. A total of 37 genes, including 21 candidate genes and 16 predicted genes, were strongly correlated ($r^2>0.50$) with different femoral neck phenotypes and prioritized for further analysis. Analysis of the related pathways among prioritized candidate genes based on their molecular function, biological process and cellular component of the gene product by Ingenuity pathway analysis revealed several direct or indirect relationships among the candidate genes related to bone metabolism including pathways related to beta-estradiol, interleukin 6, insulin growth factor 2, androgen receptor and tumor necrosis factor. This study may provide a basis for identifying the genetic determinants of skeletal traits at the hip and may lead to novel approaches to prevent and treatment of hip fracture.

***Disclosures: I. Alam**, None.*
This study received funding from: NIH.

SA262

Recurring Mutation Causing Severe/Lethal Recessive Type VIII Osteogenesis Imperfecta in African-Americans Originated in West Africa More than 300 Years Ago. W. A. Cabral*[1], A. M. Barnes*[1], C. N. Rotimi*[2], F. D. Porter*[3], J. Bailey-Wilson*[4], L. Brody*[5], J. C. Marini[1]. [1]Bone & Extracellular Matrix Branch/NICHD/NIH, Bethesda, MD, USA, [2]Inherited Disease Research Branch/NHGRI/NIH, Bethesda, MD, USA, [3]Heritable Disorders Branch/NICHD/NIH, Bethesda, MD, USA, [4]Inherited Disease Research Branch/NHGRI/NIH, Baltimore, MD, USA, [5]Genome Technology Branch/NHGRI/NIH, Bethesda, MD, USA.

Recessive Osteogenesis imperfecta (OI) is caused by defects in the genes encoding cartilage-associated protein (*CRTAP*) or prolyl 3-hydroxylase 1 (*LEPRE1*) and accounts for approximately 5% of OI cases in North America. Patients deficient in prolyl 3-hydroxylase 1 have severe to lethal bone dysplasia characterized by white sclerae and extreme osteopenia, with DEXA z-scores below -6. Although recessive OI accounts for only a small percentage of OI cases, we have identified a recurring mutation in the *LEPRE1* gene, IVS5+1G>T (Nat Genet 39:359-365, 2007). The common mutation occurs in a compound heterozygous or homozygous state in 6 of 9 probands (9 of 18 alleles) with recessive type VIII OI (OMIM #610915). All six probands with this mutation were born to carrier parents of West-African (Nigerian or Ghanaian) or African-American descent, suggesting the existence of a stable mutant allele in this population. In order to determine the carrier frequency of this mutation in African-Americans, we screened genomic DNA from unrelated African-American newborns from Pennsylvania (n=1429) and Washington, D.C. (n=588). We identified 5 carriers from Pennsylvania and 5 carriers from the District of Columbia, predicting a carrier frequency of 1 in 118-286 African-American newborns (0.35-0.85%). Our results predict a 1 in 55,000-330,000 rate of occurrence of lethal type VIII OI in African-Americans due to homozygosity for *LEPRE1* IVS5+1G>T. To verify the origin of the mutation, genomic DNA from 1097 contemporary West Africans was screened using a custom SNP assay, followed by PCR confirmation of positive samples. Fifteen of 1097 unrelated individuals (1.37%) from Nigeria and Ghana were heterozygous for *LEPRE1* IVS5+1G>T, two-thirds of whom are from the Yoruba or Ibo tribes of Nigeria. This high carrier frequency suggests that the incidence of recessive type VIII OI in West Africa from this founder mutation alone is 1 in 21,000 individuals, which is equivalent to the incidence of dominant OI in North America. Screening of microsatellites covering a 4.2Mb region surrounding the *LEPRE1* gene on chromosome 1p33-34.3 revealed a conserved haplotype of less than 500Kb in probands and carriers. These data support the occurrence of a common founder mutation over 300 years ago, consistent with our hypothesis that this West African allele was transported to the Americas via the colonial slave trade.

***Disclosures: W.A. Cabral**, None.*
This study received funding from: NIH intramural funding.

SA263

Genome-Wide Association Study Reveals Genetic Variants Associated with Bone Mineral Density, Osteoporosis and Osteoporotic Fractures. J. Richards*[1], F. Rivadeneira*[2], M. Inouye*[3], T. Pastinen*[4], N. Soranzo*[3], S. G. Wilson*[5], T. Andrew*[1], M. Falchi*[1], R. Gwilliam*[3], K. R. Ahmadi*[1], P. Arp*[2], A. M. Valdes*[1], P. Whittaker*[2], D. J. Verlaan*[6], M. Jhamai*[2], V. Kumanduri*[2], J. B. van Meurs*[2], A. Hofman*[2], H. P. Pols*[2], D. Hart*[1], G. Zhai*[1], B. H. Mullin*[5], P. Deloukas*[3], A. G. Uitterlinden*[2], T. D. Spector*[1]. [1]Twin Research Unit, King's College London, London, United Kingdom, [2]Erasmus MC, Rotterdam, Netherlands, [3]Sanger Institute, Cambridge, United Kingdom, [4]McGill University, Montreal, QC, Canada, [5]University of Western Australia, Perth, Australia, [6]McGill, Montreal, QC, Canada.

Background: Osteoporosis is diagnosed by the measurement of bone mineral density (BMD)-a highly heritable and multifactorial trait. We undertook a genome-wide association (GWA) study for BMD.
Methods: Using 314,075 single nucleotide polymorphisms (SNPs) we identified the most promising SNPs in 2,094 women from the United Kingdom (TwinsUK discovery cohort). These were then tested for replication in 6,463 individuals from three cohorts (the Rotterdam study, the Chingford study and TwinsUK replication cohort). We also investigated allelic expression levels in lymphoblast cell lines (LCLs). Replicated SNPs were tested for their association with osteoporotic fracture in the Chingford study (n = 718) and the Rotterdam study (n = 5921).
Findings: A non-synonymous SNP in the LRP5 gene was associated with decreased BMD (rs3736228: P = 6.3 x 10-12 for lumbar spine (LS) and 1.9x10-4 for femoral neck (FN)) and an increased risk of both osteoporotic fracture (odds ratio (OR) = 1.3, P = 2.0x10-3) and osteoporosis (OR = 1.3 P = 8.0x10-3). Three SNPs near the TNFRSF11B (osteoprotegerin) gene, were associated with decreased BMD (top SNP, rs4355801: P = 7.6 x10-10, P = 3.3x10-8 for LS and FN, respectively) and increased risk of osteoporosis (OR = 1.2, P = 0.038). The risk allele at rs4355801 was associated with a 2-fold decreased expression of TNFRSF11B in LCLs (P = 3.0x10-6). 22% of the entire population was at least heterozygous for both risk alleles and these alleles had a cumulative association with BMD (P for trend = 2.5 x 10-17). The presence of both risk alleles increased the risk of osteoporotic fracture (OR = 1.3, P = 6.0x10-3) and this effect was independent of BMD.
Interpretation
This first comprehensive interrogation of common genetic variants influencing BMD, in 8,557 individuals, has identified two gene variants of key biologic proteins which increase the risk of osteoporosis and osteoporotic fracture. These risk alleles, whose combined effect on fracture is similar to that of most well-replicated environmental risk factors, are present in more than one in five Caucasians.

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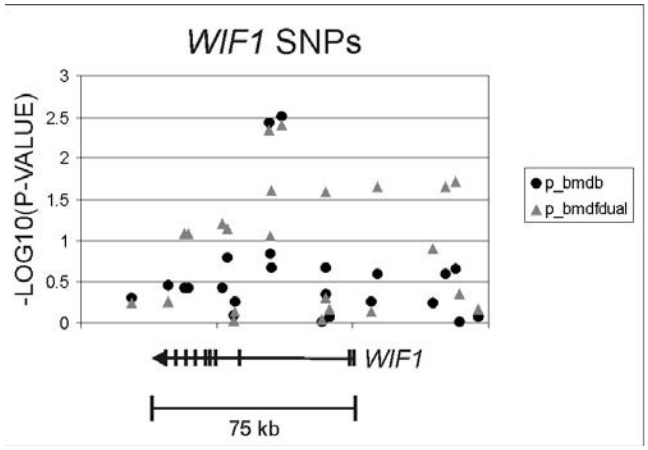
SA264

Polymorphisms in the Wnt Inhibitory Factor 1 Gene (WIF1) Are Associated with BMD in Elderly Swedish Women. C. J. Rubin[1], K. Michaelsson*[2], H. Melhus*[1], Ö. Ljunggren[1], L. Lind*[1], A. Kindmark[1]. [1]Dept. Med. Sciences, Uppsala University, Uppsala, Sweden, [2]Dept. Surgical Sciences, Uppsala University, Uppsala, Sweden.

Canonical wnt-signaling promotes bone accrual and perturbations in the wnt signaling pathway can cause osteopetrosis as well as osteoporosis (LRP5 mutations). Wnt Inhibitory Factor 1 (WIF1) is a secreted protein that inhibits wnt-proteins from binding to their receptors. In prior studies of an intercross between domestic and wild-type chicken we have observed differential expression of WIF1 between chicken with high and low BMD and have also identified a Quantitative Trait Locus (QTL) for BMD in the region where WIF1 is located.
Altogether, the data from the chicken studies and the importance of wnt-signaling in bone metabolism makes WIF1 an interesting candidate gene to explore in relation to inter-individual variation in bone traits. In the present study we genotyped 24 common single nucleotide polymorphisms (SNPs) located downstream, within and upstream of the human WIF1 gene and tested if any of these were associated with bone traits. The analyzed population-based cohort (the PIVUS study) comprises 1000 men and women, phenotyped by DXA at approximately 70 years of age.
Female-specific analysis with body mass index (BMI) and age included as covariates revealed associations between BMD (total body and total hip) and genotypes of two SNPs (Figure 1). These two SNPs (rs7309476 and rs10878228) had minor allele frequencies of 0.42 and female C-allele carriers had 6% higher total hip BMD and 3% higher total body BMD compared to female A-allele homozygotes. In addition to these, several other SNPs obtained P-values < 0.05 and may also be interesting to study in follow up studies in other cohorts.



***Disclosures:* C.J. Rubin**, *None.*

SA265

See Friday Plenary number F265.

SA266

Polymorphisms in *CYP24A1*, the Gene Encoding 25-hydroxyvitamin D-24-hydroxylase Are Associated with BMD. E. A. Streeten, J. Liu*, X. Shi*, K. Ryan*, D. McBride*, B. Mitchell, A. R. Shuldiner*. Endocrinology, Diabetes and Nutrition, University of Maryland School of Medicine, Baltimore, MD, USA.

Vitamin D deficiency is an important contributor to reduced bone mineral density (BMD). We and others have found that serum level of 25(OH)D is hereditary. Since the main source of vitamin D is endogenous production, we hypothesized that variants in the genes encoding the vitamin D synthetic *(CYP2R1, CYP27B1)* and inactivating enzymes (*CYP24A1)* are associated with BMD. The *purpose* of this study was to perform association studies between SNPs of these 3 genes and BMD.
Healthy Amish participants (n=1467), including 643 men and 843 women, mean (±SD) age of 55.4 ± 14.7 were studied at the Amish Research Clinic in Lancaster, PA. DXA was performed.(Hologic 4500) of the spine (L1-4), hip (total, TH; femoral neck, FN) and distal radius (mid, MRad; total, TRad; ultradistal, UD). Lab studies included serum calcium, PTH and 25(OH)D. Association studies were done between BMD and SNPs on 500K genome-wide association (GWAS) chips for the 3 enzymes and also between BMD and 21 haplotype-tagging SNPs from in *CYP24A1*.
Results showed T-scores: L1-4 -1.13 ± 1.38; TH -0.24 ± 1.07; FN -0.54 ± 1.15, and mean 25(OH)D 22.8 ± 7.6. From GWAS 500K chips, genotypes of 4 of 6 SNPs of *CYP24A1* were associated with BMD in 634 participants, best for SNP rs1570669 for TH (p= 0.005). One SNP of *CYP2R1*, A2293926 was associated with UD BMD (p= 0.007) and two SNPs of *CYP27B1* were associated with TRad (best p= 0.024 for A 2216496). Findings in *CYP24A1* were replicated in an additional sample of 833 Amish (total n=1467). Genotyping of 21 additional SNPS to cover the *CYP24A1* gene in 634 subjects revealed significant associations between 13 SNPs and BMD, with best associations seen in those over age 50 (below). Serum 25(OH)D, PTH, and calcium (n= 993) were not associated with genotype for SNPs of *CYP24A1*.
We *conclude* that variants of *CYP24A1*, the gene for 24-hydroxylase, which catabolizes 25(OH)D into inactive 24,25$(OH)_2$D, are associated with BMD in men and women, most strongly in those over age 50. Only minimal association was seen in those under age 50. We speculate that with reduced endogenous skin production of D_3 that occurs with aging, the relative activity of the catabolic 24-hydroxylase enzyme may become more important to maintain optimal calcitriol levels and bone health.
SNP rs3787555 (left) SNP rs1570669 (from GWAS) (right)

	Spine	Tot Hip	Fem N	Tot Rad	Mid Rad	Spine	Tot Hip	Fem N	Total Rad	Mid Rad
All (n=634)	0.005	0.015	0.015	0.001	0.002	0.033	0.004	0.009	0.021	0.035
Women (n=338)	0.048	0.004	0.004	0.034	0.032	0.077	0.012	0.12	0.156	0.194
Men (n=296)	0.034	0.221	0.324	0.002	0.009	0.246	0.178	0.047	0.077	0.108
Over 50 (n=400	0.001	0.017	0.014	0.083	0.134	0.015	0.0005	0.004	0.005	0.007
≤50 (n=234)	0.55	0.417	0.593	0.082	0.114	0.033	0.053	0.021	0.09	0.101

***Disclosures:* E.A. Streeten**, *None.*

SA267

Genome-wide Association Study Identified Novel Susceptibility Loci for Osteoporosis. Y. Guo*[1], J. T. Wang*[1], T. L. Yang*[1], F. Pan*[1], F. Zhang*[1], Z. X. Zhang*[1], X. G. Liu*[1], Q. Zhou*[1], B. Drees*[2], J. Hamilton*[2], C. J. Papasian*[2], R. R. Recker[3], H. W. Deng[2]. [1]School of Life Science and Technology, Xi'an Jiaotong University, Xi'an, China, [2]School of Medicine, University of Missouri - Kansas City, Kansas City, MO, USA, [3]Osteoporosis Research Center, Creighton University, Omaha, NE, USA.

Osteoporosis is a major public health problem. It is mainly characterized by low bone mineral density (BMD) and/or low-trauma osteoporotic fractures (OF), both of which have strong genetic determination, but specific genes are largely unknown. Using the Affymetrix 500K array set, we performed a case-control genome-wide association study in 700 elderly Chinese Han subjects (350 with osteoporotic hip fractures and 350 healthy matched controls). For the significant SNPs identified for OF, we further examined their relevance with hip BMD in both Chinese and Caucasian populations involving an addition 7,009 individuals. We found three novel loci highly significantly associated with OF, including rs13182402 within *ALDH7A1* ($P = 3.09\times10^{-9}$, odds ratio = 2.92), rs6711417 near *UBR3* ($P = 4.61\times10^{-6}$, odds ratio = 0.58), and rs6064822 within *PHACTR3* ($P = 2.36\times10^{-5}$, odds ratio = 0.55). Especially, these three loci were unequivocally confirmed as significantly associated with hip BMD even across ethnic boundary, in both Chinese and Caucasians (rs13182402: combined $P = 8.18\times10^{-5}$; rs6711417: combined $P = 3.60\times10^{-5}$; rs6064822: combined $P = 3.80\times10^{-4}$), further attesting to their importance in osteoporosis. *ALDH7A1* gene degrades and detoxifies acetaldehyde, which inhibits the osteoblast proliferation and result in the decreased bone formation. *UBR3* encodes E3 ubiquitin ligase, which plays a specific role in osteoblast differentiation. *PHACTR3* is involved in protein phosphatase pathway regulating diverse cellular processes. Our findings provide a new insight into the pathogenesis of osteoporosis, which warrant further studies to explore generality in other populations, their biological effects and functional mechanisms.

***Disclosures:* *Y. Guo**, None.*

SA268

A Common Single Nucleotide Polymorphism in the High Mobility Group AT-hook 2 (*HMGA2*) Gene Is Associated with Bone Mass in Men. A. L. Kuipers[1], J. A. Cauley[1], K. Ensrud[2], C. L. Gordon[3], D. Inglis*[3], V. W. Wheeler*[4], A. L. Patrick*[4], C. H. Bunker*[1], C. S. Nestlerode*[1], A. R. Hoffman*[5], E. S. Orwoll[6], J. M. Zmuda[1]. [1]Epidemiology, University of Pittsburgh, Pittsburgh, PA, USA, [2]VA Medical Center and University of Minnesota, Minneapolis, MN, USA, [3]McMaster University, Hamilton, ON, Canada, [4]Tobago Health Studies Office, Sarborough, Trinidad and Tobago, [5]VA Palo Alto Health Care System and Stanford University Medical Center, Palo Alto, CA, USA, [6]Oregon Health and Science University, Portland, OR, USA.

HMGA2 encodes a non-histone, chromosome protein belonging to the high mobility group (HMG) family that regulates transcription of target genes. Knockout mouse experiments and a case report of a boy with a constitutional chromosomal rearrangement suggest that HMGA2 plays a role in growth regulation. Moreover, a recent genome-wide analysis (GWA) identified a novel association of the *HMGA2* gene with human stature. To further evaluate this gene and skeletal measures, we genotyped a C-T polymorphism in the 3' UTR of *HMGA2* identified from the GWA of short stature, and conducted genetic association analysis with body size and bone mass in an initial discovery population consisting of 1,813 men of African ancestry and in a validation sample of 979 Caucasian American men. The mean age of these men were 58.9±10.4 years and 73.7±5.8 years, respectively. Skeletal measurements were obtained with peripheral quantitative computed tomography (pQCT). Specialized segmentation and analysis software (pQCT Pro) was also used to assess apparent trabecular architecture in the African ancestry men. The minor T allele frequency was 0.34 in African and 0.49 in Caucasian men. There was no significant association with height or body weight in either ethnic group. However, there was an additive association with BMD where the T allele was associated with lower trabecular volumetric BMD at the tibia (P=0.002 in Caucasians; P=0.007 in Africans). For example, African ancestry men with the T/T genotype had 4% lower trabecular BMD compared to men with the C/C genotype (231±2 vs 221±3 mg/cm^3), whereas, the heterozygotes had intermediate values (227±2 mg/cm^3). Apparent trabecular thickness was 9% less (P=0.005) and apparent trabecular number was 15% greater (P=0.01) among African ancestry men with the T/T than C/C genotypes. Our analysis suggests a novel association between a common *HMGA2* genetic variant and trabecular bone mass and structure in older men.

***Disclosures:* *A.L. Kuipers**, None.*
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SA269

Osteosclerotic Prostate Cancer Metastasis to Murine Bone: Enhanced with Increased Bone Turnover. P. C. Buttke*[1], E. M. Paul*[1], R. A. Sikes*[2], R. R. Gomes, Jr[1]. [1]Orthopaedics and Rehabilitation, H089, Penn State College of Medicine, Hershey, PA, USA, [2]Biological Sciences, University of Delaware, Newark, DE, USA.

Spontaneous development of osteoblastic lesions of prostate cancer (PCa) in mice is modeled by orthotopic (prostatic) deposition of neoplastic cells followed by an extremely long latency with associated low incidence of bone metastasis. Intracardial injection results in overt bone metastases only with osteoclastic PCa cells (i.e., PC-3). Herein, we report that androgen independent osteoblastic PCa cells will readily colonize bone when it is in a high remodeling state. SCID/bg mice were subjected to periods of intermittent parathyroid hormone (PTH) exposure, followed by an intracardiac infusion of C4-2 PCa cells. Analysis of bone turnover markers in serum from mice treated intermittently with PTH revealed significant increases in osteocalcin (55.06±7.5 vs 74.01±18.5 ng/ml) and TRACP-5b (3.3±0.6 vs 4.81±0.8 U/L), but no change in type I collagen CTX levels relative to control mice. Subsequent MicroCT analysis of femurs revealed significant increases in bone mineral density, trabecular thickness (0.056±0.002 vs 0.062± 0.001 μm) and porosity but significant decreases in connectivity density and trabecular number (5.756±0.17 vs 5.02± 0.20 mm-1) in mice treated with PTH relative to controls. By eight weeks post-PCa-injection 70% of mice, pre-treated with PTH, demonstrated detectable serum prostate specific antigens (PSAs). Immunohistochemical labeling of femurs for PSA and pan-Cytokeratin revealed the presence of tumor cell nests, as opposed to single cells, in marrow and trabecular spaces. These results suggest that 1) mouse bone physiology is an important factor for developing osteoblastic/sclerotic PCa bone metastases in murine hosts; 2) the establishment of osteosclerotic prostate cancer bone metastases in mice is enhanced by alterations in bone turnover, and 3) regimens of PTH administration for osteoporosis may require caution and/or enhanced monitoring of cancer patients at risk for developing bone metastases.

***Disclosures:* *P.C. Buttke**, None.*
This study received funding from: Pennsylvania Department of Health.

SA270

See Friday Plenary number F270.

SA271

Strength Training Prevents Bone Loss at the Spine in Older Breast Cancer Survivors: Preliminary Findings from an RCT. K. M. Winters-Stone*[1], J. A. Bennett*[1], A. Reiner*[1], J. Dobek*[1], A. Schwartz*[2], L. Nail*[1]. [1]School of Nursing, Oregon Health & Science University, Portland, OR, USA, [2]School of Nursing, Arizona State University, Tempe, AZ, USA.

Approximately 85% of women who receive a first diagnosis of breast cancer are aged 50 and over, thus the combined effect of aging, menopause and breast cancer treatment may threaten bone health in these women. Yet, few studies have focused on evaluating how exercise interventions, particularly resistance training, benefit older breast cancer survivors. PURPOSE: The purpose of this report is to present preliminary findings for an on-going resistance training study in older breast cancer survivors. METHODS: We are conducting a 12-month randomized controlled trial of resistance (strength) training (STR) versus a control group of flexibility (FLEX) training in early-stage, older BCS (mean age: 63.7 yrs) who are > 1 year past radiation and/or chemotherapy. To date, 31 women (STR n=20, FLEX n=11) have completed 12 months of exercise training and interim testing. Interim findings for bone mineral density of the total hip and lumbar spine assessed by DXA are presented. Within group changes were determined by paired t-tests and between group changes were determined by repeated measures ANOVA. RESULTS: After 12 months of exercise, spine and hip BMD was maintained in STR (0.3% and -0.8%, respectively) compared to significant decreases at both sites in FLEX (-1.7% and -1.6%, respectively). Between group changes were only significant for spine BMD (p<.03). CONCLUSIONS: These preliminary data show trends that resistance exercise maintains BMD at the spine compared to a control program of flexibility exercise in older breast cancer survivors. Definitive conclusions must await completion of the full trial.

***Disclosures:* *K.M. Winters-Stone**, None.*
This study received funding from: Susan G. Komen for the Cure Foundation.

SA272

See Friday Plenary number F272.

SA273

TGF-β Activates Prostate Cancer Bone Metastases, Pro-Osteolytic Gene Expression and the New TGF-β Signaling Regulator PMEPA1. P. G. J. Fournier, G. A. Clines, J. M. Chirgwin, T. A. Guise. Endocrinology, University of Virginia, Charlottesville, VA, USA.

Transforming growth factor-β (TGF-β) increases breast cancer and melanoma bone metastases by activating pro-osteolytic genes (PTHrP, IL-11 or CTGF). We hypothesized that TGF-β would also promote prostate cancer bone metastases.
A specific inhibitor of the TGF-β type I receptor kinase, SD-208, decreased Smad2 phosphorylation induced by TGF-β in PC-3 human prostate cancer cells. Osteolytic bone metastases were caused by inoculating PC-3 cells in the left cardiac ventricle of athymic mice. SD-208 (50mg/kg/d po) begun at the time of tumor inoculation decreased osteolytic bone metastases (56% decrease, $P<0.05$) and increased survival (57 to 69 days median survival, $P<0.05$). When treatment began after osteolytic lesions were detected, SD-208 decreased bone destruction (47% decrease, $P<0.05$) without significantly improving survival (51 to 55 days, $P>0.1$).
We analyzed PC-3 cells treated ± TGF-β (5ng/mL) by Affimetrix gene array and sqRT-PCR. Significantly up-regulated genes included PTHrP, IL-11, CTGF and ADAM19, which can increase bone resorption, as well as MMP-13 and thrombospondin-1, two activators of TGF-β.
The most increased gene was PMEPA1 (>20x), a protein highly expressed in breast, colon and prostate cancers. sqRT-PCR of mRNA from PC-3 cells treated ±TGF-β and ±cycloheximide or ±actinomycin-D showed that TGF-β directly controls PMEPA1 transcription. Dual-luciferase experiments showed that a 3.7kb fragment of PMEPA1 promoter is activated by TGF-β. This fragment contains 5 consensus Smad binding elements, but mutagenesis showed that they are not involved in TGF-β induction. Analysis of promoter deletion constructs and overexpression of Smad2, 3 and 4 demonstrate that TGF-β induction of PMEPA1 promoter is mediated by a 1.2kb proximal fragment through both Smad and non-Smad mechanisms.
Western blotting confirmed that TGF-β quickly and dose-dependently increased the cytosolic isoform of PMEPA1 in PC-3 cells. PMEPA1 proteins contain 2 PPxY domains that can interact with Smurf ubiquitin ligases (which can regulate degradation of Smads and type I TGF-β receptor) and a Smad-interaction motif (found in DNA binding co-factors that control the affinity of Smads for DNA), suggesting that PMEPA1 regulates TGF-β signaling. shRNA knockdown of PMEPA1 significantly decreased TGF-β activation of the Smad reporter $(CAGA)_9$. PMEPA1 induced by bone-derived TGF-β could potentiate TGF-β signaling in cancer cells.
Our results show that TGF-β can promote PC-3 bone metastases through a transcriptional program that increases bone resorption and potentiates TGF-β signaling.

***Disclosures: P.G.J. Fournier**, None.*
This study received funding from: DoD PCRP, NIH, Mellon Prostate Cancer Institute.

SA274

TGF-β Blockade Inhibits Osteolytic but not Osteoblastic Prostate Cancer Metastases. P. G. J. Fournier, K. S. Mohammad, C. R. McKenna*, X. Peng*, J. M. Chirgwin, T. A. Guise. Endocrinology, University of Virginia, Charlottesville, VA, USA.

Prostate cancer cells frequently metastasize to bone causing bone destruction and/or formation. Bone-derived transforming growth factor-β (TGF-β) enhances osteolytic metastases. Inhibition of TGF-β signaling with SD-208, a kinase inhibitor of the TGF-β type I receptor, decreased osteolytic metastases in breast cancer and melanoma models. The drug also increased bone mineral density by stimulating osteoblastic bone formation and inhibiting osteoclastic bone resorption, suggesting that it might be unsuitable against osteoblastic metastases, which are common in prostate cancer. Here we report the effects of SD-208 in mouse models of osteolytic and osteoblastic prostate cancer bone metastases.
Male *nude* mice were inoculated in the left cardiac ventricle with osteolytic PC-3 cells (10^5 cells, n=11-14/group). Immunostaining of sections of PC-3 bone metastases showed nuclear localization of phosphorylated Smad2, a marker of activated TGF-β signaling. Mice were treated with SD-208 (50mg/kg/d po) or vehicle and followed by x-ray. SD-208 did not decrease bone metastasis number compared to vehicle (vehicle 10/11 vs SD-208 12/14) but did decrease bone lesion area measured by x-ray compared to vehicle (6.7±3.3mm² vs 15.3±2.8mm², $P<0.05$) and increased mouse median survival (57 to 69 days, $P<0.05$).
In a pilot experiment, we inoculated cells from the LuCaP 23.1 human prostate cancer osteoblastic xenograft into the tibias of *nude* mice ($2x10^5$ cells, n=3-5/group). Nuclear immunostaining of phosphorylated Smad2 demonstrated active TGF-β signaling in tumor cells at sites of bone metastases. However, SD-208 treatment decreased neither incidence of metastases nor skeletal tumor burden, measured by histomorphometry compared to vehicle-treated mice. New bone formation in the tibias containing tumor was not augmented by SD-208 treatment, while trabecular bone measured in vertebrae free of tumor cells was increased by SD-208 (BV/TV 270% vs vehicle, $P<0.01$).
Our results show that blockade of TGF-β signaling with SD-208 increases bone formation, while the drug does not enhance skeletal lesions in a model of osteoblastic prostate bone metastases. SD-208 does inhibit osteolytic metastases from prostate cancer cells. Overall, TGF-β blockade should be an efficient therapy against bone metastases with osteolytic and mixed bone responses to tumor but less so against purely osteoblastic lesions.

***Disclosures: P.G.J. Fournier**, None.*

SA275

See Friday Plenary number F275.

SA276

Breast Cancer Cell Conditioned Media Stimulates Osteoblastic Cells to Attract Breast Cancer Cells. K. P. Chin-quee*, H. J. Donahue. Department of Orthopaedics and Rehabilitation, Penn State College of Medicine, Hershey, PA, USA.

Breast cancer owes most of its mortality and morbidity to its metastasis to distant sites, especially bone. While a better understanding of what cancer cells do when they colonize bone is emerging (i.e. the "vicious cycle"), there is relatively little known as to why breast cancer cells preferentially metastasize to bone. To address this issue, we examined the hypothesis that secreted factors from breast cancer cells induce changes in osteoblastic cells, which cause them to attract metastasizing breast cancer cells. We focused on MDA-MET, a breast cancer cell line derived from MDA-MB-231, created by an *in vivo* selection process leading to a highly bone specific metastasis, and hFOB 1.19, a human fetal osteoblastic cell line. hFOB 1.19 cells were incubated for 24hr in conditioned media from MDA-MET cells (group 1), HTERT-HME1 cells (normal breast epithelial; group 2), or hFOB 1.19 cells (group 3). After this incubation, treatment media was removed from the hFOB 1.19 cells, the cells were washed with PBS and serum free media added. At the end of 12hr, this serum-free media, from all three groups was collected. The control group (group 4) consisted of serum-free media unexposed to any cells. To test the chemoattractant ability of the collected media from these four groups, we used a transwell cell migration assay, where migratory cells are placed in removable transwell inserts, the bottom of which consists of porous membranes (pore size 8μm), in direct contact with the test media in the lower well. The ability of media from each group to attract the MDA-MET cells, causing them to migrate to the other side of the membrane, was examined. The number of migratory cells was quantified by fluorescent labeling of the lysed cells, given in fluorescent units. Data are represented as mean flurorescence ± SEM. We found a greater migration of breast cancer cells toward media from group 1 than toward either group 2 or group 3 (1533±58.2 vs 957±140 vs 983±51.4), respectively; $p< 0.01$), but no difference between migration towards group 2 (treated by HTERT-HME1) and group 3 (untreated) media. In addition, Group 3 media from untreated hFOB 1.19 cells caused a greater migration of cells than group 4, serum -free media alone (983±51.4 vs 653±56.1; $p<0.01$). Our results suggest that factors constitutively secreted by bone cells attract breast cancer cells. More importantly, metastatic breast cancer cells (MDA-MET) but not normal breast epithelial cells (HTERT-HME1), secrete factors, which in turn increase the ability of osteoblastic cells to attract breast cancer cells.

***Disclosures: K.P. Chin-quee**, None.*

SA277

See Friday Plenary number F277.

SA278

Osteoclast Precursors Play a Role in Recruiting Breast Cancer Cells and Osteoblasts onto the Bone in Vitro. Z. Shi*, X. Feng. Pathology, Univ. of Alabama at Birmingham, Birmingham, AL, USA.

Breast cancer (BC)-induced osteolysis is not only a consequence but, more devastatingly, also a cause of BC skeletal metastasis. Three key players, osteoclasts (OC), BC cells, and osteoblasts (OB), are involved in BC-induced osteolysis. Indeed, BC cells express PTHrP, which stimulates the production of RANKL by OB. The produced RANKL promotes the differentiation of OC precursors into mature OC to resorb bone, resulting in the release and activation of TGF-β embedded in the bone matrix. The released TGF-β further stimulates the expression of PTHrP. As such, BC-induced osteolysis is a vicious cycle. Although this mode of the action is well documented, it is unclear how BC cells and OB are brought to the scene to act. Specifically, in tumor-induced-osteolysis, before the onset of bone resorption, OB and BC cells must be recruited to bone surface to produce RANKL, which is required for OC formation. However, based on the current models, these cells are attracted by factors released from bone matrix by OC, but how OC formed in the first place is unknown. In this study, we propose a new hypothesis that OC precursors play a key role in OB and BC recruitment to bone. We performed chemotaxis assays with the following conditioned media (CM): CM1 - from OC precursors cultured in Teflon beaker, CM2 - from the OC precursors cultured in tissue culture plates without bones slices and CM3 - from the OC precursors cultured in tissue culture plates with bones slices. CM were all prepared using MEM containing 0.2 % FBS. The control medium is MEM containing 0.2% FBS. 600ul CM was added to one well in Transwell. BC cells (MDA-MB-231) or OB (2×10^6/ml) were in MEM with 0.2% FBS. Transwell were inserted into well and 100ul cells (2×10^5 each insert) were added to the inside compartment. Transwell were cultured for 4 hours at 37°C and 5% CO_2. After 4 hours, cells were fixed for 30 seconds. Cells were stained for one hour with Crystal Blue and then counted. The results indicate that all three CM (CM 1, CM2 and CM3) exhibited chemotaxis on MDA-MB-231 cells compared with control medium. More importantly, CM3 demonstrated a higher capacity in inducing chemotaxis on MDA-MB-231 cells than CM1 and CM2. Moreover, all three CM (CM 1, CM2 and CM3) exerted higher chemotaxis on OB compared to control medium. These data indicate that OC precursors can exert chemotatic effects on BC cells and OB, suggesting that OC precursors play a key role in BC bone metastasis by recruiting OB and BC cells to the active bone remodeling sites. These studies have not only deepened our understanding of the mechanisms underlying BC cells/OB recruitment to bone but also opened new directions for future investigation of the mechanism underlying BC bone metastasis.

***Disclosures: Z. Shi**, None.*

SA279

See Friday Plenary number F279.

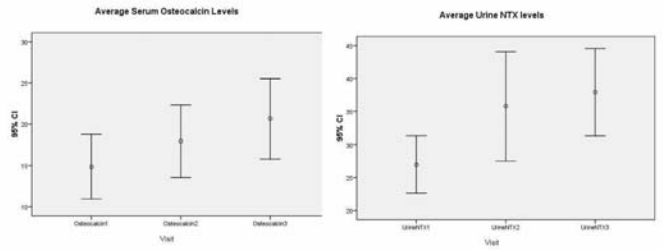
SA280

Effects of Androgen Deprivation Therapy on Bone Health in Prostate Cancer. A. K. McDonough[1], G. G. Teng*[2], J. Abott*[1], R. Nair*[1], C. Amling*[3], J. Colli*[4], J. B. Fiveash*[5], R. Lopez*[6], D. Urban*[3], J. R. Curtis[1], K. Saag[1]. [1]Division of Clinical Immunology and Rheumatology, University of Alabama at Birmingham, Birmingham, AL, USA, [2]Division of Rheumatology, National University Hospital, Singapore, Singapore, [3]Division of Urology, University of Alabama at Birmingham, Birmingham, AL, USA, [4]Birmingham VA Medical Center, Birmingham, AL, USA, [5]Department of Radiation Oncology, University of Alabama at Birmingham, Birmingham, AL, USA, [6]Department of Radiology, University of Alabama at Birmingham, Birmingham, AL, USA.

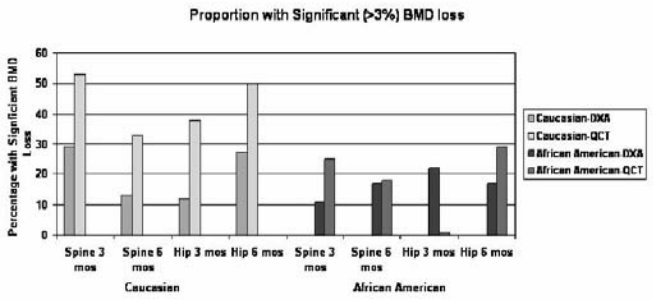
Androgen deprivation therapy (ADT) can cause bone loss in men but it is uncertain how rapidly this develops and if its bone effects differ by race/ethnicity. We examine whether short-term ADT leads to significant increases in bone turnover markers (BTM) or a decline in BMD over 6 months. We also determined the sensitivity of QCT compared with DXA for identifying early BMD change.

Men > 40 yrs with M0 prostate cancer were enrolled within 1 month of ADT initiation and seen at baseline, 3, and 6 mos. Patients with other causes of metabolic bone disease or on anti-osteoporotic meds were excluded. Descriptive analyses examined trends in osteocalcin, urine NTX, and BMD. We also analyzed factors associated with BTM change and BMD loss.

Patients (n = 30) were 40% African American (AA), had mean age 69.5 ± 8.9 yrs and mean BMI 27.6 ± 4.3. We found significant increases in BTM (p = 0.001) as early as 3 months into ADT.



Non-statistically significant trends in BMD suggest more bone loss among Caucasians by QCT and DXA. No significant association with BMD or BTM was seen with alcohol intake, smoking, BMI, testosterone levels, or caffeine. QCT and DXA BMD of the hip (r = 0.71) and spine (r = 0.87) highly correlated.



In summary, ADT significantly increased BTM within 6 months of initiating therapy. Trends in BMD decline were notable in Caucasian, but not African Americans. QCT appears more sensitive than DXA to identify need for early intervention for men who are may have accelerated ADT-associated bone loss.

***Disclosures:* A.K. McDonough**, *None.*
This study received funding from: TAP Pharmaceutical.

SA281

See Friday Plenary number F281.

SA282

Quantitative Image Analysis Method for Measuring Whole-body Tumor Burden in a Mouse Model of Breast Cancer Bone Metastasis. R. S. Käkönen*, J. P. Rissanen, M. I. Suominen, J. M. Halleen. Pharmatest Services Ltd, Turku, Finland.

Non-invasive fluorescence imaging is a promising new tool with several advantages over traditional radiography and histology; it is fast and allows whole-body imaging without the need of sacrificing the animals. However, the means of transforming the imaging output into descriptive and reliable quantitative data have been limited. The most commonly used output data, fluorescence emission area as an index of tumor burden does not, however, take the fluorescence signal intensity into account. Furthermore, tumor burden is conventionally measured only in histological sections of hind limbs, whereas fluorescence imaging allows whole-body imaging, but the correlation between these two methods has been poorly studied. We have validated a new image analysis method using GFP-MDA-MB-231(SA) breast cancer cells in a mouse model of breast cancer bone metastasis. Five-week-old female athymic nude mice were inoculated intracardially with MDA-MB-231(SA) breast cancer cells transfected with pTurboGFP-N vector (Evrogen JSC, Moscow, Russia). Three study groups were included (n=12/group): 1) Vehicle, 2) Doxorubicin 2.5 mg/kg, 3) Doxorubicin 5 mg/kg (all groups administered i.p. once a week) .The tumor burden was assessed at day 14 and at the end of the study by measuring the total area and signal intensity emitted by GFP-MDA-MB-231(SA) cells. Osteolytic lesion area was measured on day 14 and at the end of the study by radiography. At necropsy, all macroscopic signs were recorded and tissue samples were collected for histology. Based on our earlier in vitro and in vivo studies, GFP-MDA-MB-231(SA) cells retained similar tumor growth profile and metastatic characteristics as parental cells. To measure the tumor burden by fluorescent imaging we created a value called tumor burden index which takes into account both the signal area and intensity emitted by the cancer cells transfected with GFP. The development of osteolytic lesions and tumor area was inhibited by doxorubicin at 5mg/kg. This anti-tumor effect of doxorubicin was more prominent when the tumor area was measured using the tumor burden index compared to conventional histological methods. Furthermore, the tumor burden index correlated well with histological analysis of tumor burden at the sites where histological analyses were performed. These results suggest that GFP imaging is a fast and reliable tool that can be used as quantitative method for assessment of whole-body tumor burden in a mouse model of breast cancer bone metastasis. This method negates the need to use laborious and time-consuming histology for assessing tumor burden.

***Disclosures:* R.S. Käkönen**, *None.*

SA283

See Friday Plenary number F283.

SA284

Osteoblast Differentiation Induced by Shh-expressing Prostate Cancer Cells Is Enhanced by Ascorbic Acid. M. L. G. Lamm, S. M. Zunich*. Pediatrics, Northwestern University Feinberg School of Medicine, Chicago, IL, USA.

Metastatic prostate carcinoma, the major cause of mortality among men with advanced prostate cancer, occurs predominantly in bone. Identification of signaling factors that mediate prostate cancer cell-bone stroma interactions is vital to our understanding of prostate cancer metastasis in bone. Sonic hedgehog (Shh), a secreted glycopeptide, is expressed in human prostate cancer tissues and several established human prostate cancer cell lines. We have previously reported that human prostate cancer cells, LNCaP, modified to overexpress Shh (LNShh cells) directly activated the hedgehog pathway in mouse pre-osteoblast MC3T3-E1 cells and induced osteoblast differentiation, a requisite process in prostate cancer metastasis in bone. LNShh-mediated initiation of early phase osteoblast differentiation in MC3T3-E1 cells occurred in the absence of exogenous ascorbic acid (AA), a factor that is routinely added to drive osteoblast differentiation in vitro.

The purpose of the present study was to determine the role of exogenous AA in osteoblast differentiation induced by LNShh cells. In a novel co-culture system, MC3T3-E1 cells and control LNCaP or LNShh cells were co-cultured as mixed cell populations within the same culture well or chamber in the absence or presence of AA (50 microgram/ml). AA promoted the deposition of collagen, visualized by von Gieson staining, in the extracellular matrix of mixed co-cultures of MC3T3-E1 cells with either control LNCaP or LNShh cells. AA increased alkaline phosphatase activity and gene expression in mixed co-cultures of MC3T3-E1 and control LNCaP cells, and markedly enhanced Shh-stimulated alkaline phosphatase activity and gene expression in co-cultures of MC3T3-E1 and LNShh cells. Surprisingly, AA enhanced the expression of Shh signaling target genes Ptc1 and Gli1 in MC3T3-E1 cells co-cultured with LNShh but not with control LNCaP cells. To determine whether AA exerted a direct effect on the Shh pathway, MC3T3-E1 cells were cultured in the presence of Shh peptide (Shh-N) and/or AA for 24h. Data show that Shh-N, but not AA, increased Gli1 and Ptc1 expression, and there was no significant enhancement of gene levels by AA in Shh-N-treated cells.

Collectively, these data indicate that Shh and AA initiate early phase osteoblast differentiation via independent pathways. However, AA enhances Shh-mediated actions by indirectly activating the hedgehog pathway. Our studies demonstrate that paracrine signaling between prostate cancer cells and osteoblasts via the Shh pathway is sufficient to induce osteoblast differentiation, and this pathway can be modulated by other differentiation factors including ascorbic acid.

***Disclosures:* M.L.G. Lamm**, *None.*
This study received funding from: National Cancer Institute, American Cancer Society.

SA285

Inhibition of Multiple Myeloma (MM) Growth and Preservation of Bone with Combined Radiotherapy and Anti-angiogenic Agent. D. Jia[1], R. Halakatti*[1], C. Jackson*[1], L. J. Suva[2], L. Hennings*[3], X. Li[4], S. Yaccoby[4], P. M. Corry*[1], R. J. Griffin*[1]. [1]Department of Radiation Oncology, U Arkansas for Medical Sciences, Little Rock, AR, USA, [2]Department of Orthopedics, U Arkansas for Medical Sciences, Little Rock, AR, USA, [3]Department of Pathology, U Arkansas for Medical Sciences, Little Rock, AR, USA, [4]Myeloma Institute for Research and Therapy, U Arkansas for Medical Sciences, Little Rock, AR, USA.

MM is characterized by its bone marrow preference and bone destruction. Although radiotherapy has been proven effective in cancer control in general, its use in MM treatment is less than routine except in a palliative setting. The efficacy of radiation (Rad) in MM growth control and bone protection was examined in a mouse model, wherein human MM cells expressing luciferase (Luc) were injected into bone grafts pre-implanted s.c. in SCID mice. The mice were divided into 4 groups: control, anginex (Ax), Rad, and Ax+Rad. Ax, a 33 a.a. anti-angiogenic peptide, was given 3 times a wk at 20 mg/kg/day. Rad was applied to the MM-containing bone grafts in 5 Gy fractions, once per wk, 2h after the last dose of Ax for a total 40 Gy over 8 wk. MM growth was estimated by live imaging Luc activity in bone grafts, measuring the dimensions of the grafts, and quantitating human lambda light chain in serum. Bone grafts were analyzed by histology for cellular and vascular features following radiography and microCT analyses for BMD and architecture. A 114-fold increase in Luc activity over baseline was seen in control mice by wk 8, whereas in mice receiving Ax+Rad Luc activity was reduced to 50% of the baseline. Ax or Rad alone had minimal effects. Luc activity correlated with serum MM marker as well as MM outgrowth from the bone grafts as reflected by graft volume. To evaluate MM relapse following these modalities, tumor growth was monitored for an additional 4 wk after the treatments ended. Graft volume doubled to 3652 mm^3 in the controls over this post-therapy period, reached 2273 mm^3 and 775 mm^3 in Ax- and Rad-treated mice, respectively, but remained small in Ax+Rad group (207 mm^3). Consequently, bone grafts in the controls were mostly replaced by MM cells, and Ax or Rad alone did not prevent graft destruction. By contrast, vBMD as well as cortical and trabecular structure were preserved in grafts treated with Ax+Rad. Histological analysis showed that the number of MM loci, new blood vessels and bone resorbing osteoclasts were lower, while the number of cuboidal osteoblasts were higher in bone grafts receiving Ax+Rad, as compared with that in the other three groups ($p < 0.05$). Our results demonstrate that radiotherapy, when primed by drugs such as anti-angiogenic agents, can significantly control MM growth and prevent skeletal destruction in this model, thus may have potential in MM focal lesion control.

Disclosures: ***D. Jia****, None.*

SA286

See Friday Plenary number F286.

SA287

Role of Annexin II and Annexin II Receptor in Homing Multiple Myeloma Cells in the Bone Marrow. D. Del Prete*[1], G. Lu[1], F. Esteve*[1], Y. Shiozawa*[2], R. S. Taichman[2], G. D. Roodman[3]. [1]Medicine/Hem-Onc, University of Pittsburgh, Pittsburgh, PA, USA, [2]School of Dentistry, University of Michigan, Ann Arbor, MI, USA, [3]Medicine/Hem-Onc, University of Pittsburgh and VA Pittsburgh Healthcare System, Pittsburgh, PA, USA.

Annexin II (AXII) is a multi-functional protein expressed on the surface of rejuvenating cells like fibroblasts, endothelial and epithelial cells, and involved in cell proliferation, adhesion and migration. AXII is expressed by osteoblasts (OBs) and stromal cells in the bone marrow and has been shown to play a critical role in the initial adhesion of hematopoietic stem cells (HSCs) to the marrow niche (Blood 2007; 110:82-90). $AXII^{-/-}$ mice have fewer HSCs and the adhesion of HSCs to OBs derived from these mice is significantly impaired compared with OBs from wild-type animals. Because myeloma (MM) cells home to the marrow in an analogous fashion to HSC, and AXII mRNA is present in MM cells, we assessed the role of AXII and its receptor (AXIIR) in MM cell interaction with the marrow microenvironment, since the homing of MM cells to the bone marrow is critical for their survival and proliferation. We previously identified an AXIIR on human stromal cells. KM101 human stromal cells and MM.1S myeloma cells as well as primary human stromal and myeloma cells were tested. RT-PCR and Western blot analysis showed that AXII and AXIIR were expressed by all the cell types tested. However, the expression levels of AXII and AXIIR differed between stromal and myeloma cells. Stromal cells expressed high levels of AXII compared with MM cells, and MM cells expressed more AXIIR than stromal cells. Further, cocultures of MM.1S cells with KM101 cells increase AXIIR expression by MM.1S cells. To further characterize the role of AXII in the interaction of MM.1S cells with stromal cells adhesion assays were performed using an AXII-blocking peptide. Adhesion of MM.1S cells to KM101 cells was dose-dependently inhibited by the AXII peptide. These results suggest a potential critical role for AXII and AXIIR in localizing MM cells to the bone marrow.

Disclosures: ***D. Del Prete****, None.*

SA288

See Friday Plenary number F288.

SA289

Increased Signaling Through p62 in the Multiple Myeloma Microenvironment Increases Myeloma (MM) Cell Growth and Osteoclast (OCL) Formation. Y. Hiruma, N. Kurihara, G. D. Roodman. Medicine/Hem-Onc, University of Pittsburgh, Pittsburgh, PA, USA.

The bone microenvironment plays a critical role in promoting both tumor growth and bone destruction in MM. Marrow stromal cells produce factors, which stimulate both the growth of MM cells and bone destruction and are key regulators of these processes. Marrow stromal cells produce these factors in increased amounts when they bind MM cells through adhesive interactions mediated via VCAM-1 on stromal cells and β_1 integrins on MM cells. We examined the role of sequestosome-1 (p62), a recently described member of the NF-κB signaling pathway, in MM, since p62 sits at the crossroads of multiple signaling pathways potentially involved in both osteoclastogenesis and MM cell growth. Our hypothesis is that inhibiting p62 signaling will block the production of cytokines in the MM-bone microenvironment in response to the increased levels of TNF-α present in the MM microenvironment, decrease VCAM-1 expression on stromal cells, and markedly diminish osteolytic bone destruction and MM growth. Therefore, we isolated primary marrow stromal cells from MM patients and normals and measured p62 and PKC activation in MM marrow. PKC specifically interacts with p62 to increase its downstream signaling. Levels of phospho-PKC and VCAM-1 were significantly elevated in MM patients. We then blocked p62 activity in human stromal cells. p62 siRNA (40 nM) was transduced into normal and MM human stromal cells and p62 expression was decreased by at least 70% in these stromal cells. Stromal cells treated with p62siRNA or control siRNA were cultured with or without MM.1S cells for 3 days in separate experiments. The levels of VCAM-1 in p62siRNA transduced stromal cells were significantly lower than in control siRNA and untreated stromal cells. In addition, IL-6 production was increased when MM.1S myeloma cells were co-cultured with control siRNA treated stromal cells but not with p62siRNA treated stromal cells. Further, stromal cells lacking p62 minimally supported the growth of MM cells compared to control siRNA p62 containing stromal cells. Stromal cells lacking p62 also produced much lower levels of RANKL, and OCL formation was markedly decreased when these cells rather than control stromal cells were cocultured with normal OCL precursors. Studies of the mechanisms responsible for decreased IL-6 and RANKL production and VCAM-1 expression on MM stromal cells lacking p62 showed they resulted from decreased NF-κB and p38MAPK signaling. These results show that signaling through p62 plays an important role in MM cell growth and OCL formation induced by cytokines that are upregulated in the MM microenvironment.

Disclosures: ***Y. Hiruma****, None.*

SA290

Fracture Risk in Patients with Different Types of Cancer. P. Vestergaard[1], L. Rejnmark[2], L. Mosekilde[2]. [1]The Osteoporosis Clinic, Aarhus Amtssygehus, Aarhus, Denmark, [2]Department of Endocrinology and Metabolism C, Aarhus Amtssygehus, Aarhus, Denmark.

Background: Few studies on the risk of fractures in patients with cancer exist, and little is known on the mechanisms of fractures in patients with cancer. We studied the risk of fracture in patients with various types of cancer.
Subjects and methods: Case control study. There were 124,655 fracture cases and 373,962 age and gender matched controls.
Results: An increased risk of fractures, primarily within the first year after diagnosis was seen in patients with primary bone cancer (OR=3.51, 95% CI: 1.54-8.01), multiple myeloma (OR=5.21, 95% CI: 2.96-9.19), metastases to the bone (OR=5.28, 95% CI: 3.58-7.79), metastases to other organs than bone (OR=1.85, 95% CI: 1.50-2.29), lung cancer (OR=1.90, 95% CI: 1.51-2.38), and cancer of the liver, gall bladder and pancreas (2.14, 95% CI: 1.39-3.31). For patients with prostate cancer an increase in the risk of fractures was seen with time. Other cancer types were not associated with an increased risk of fractures.
Conclusions: A high risk group regarding fractures includes cancers primarily affecting the bone (primary bone cancer, multiple myeloma, metastases to the bone, metastases to other organs than bone, lung cancer, and cancer of the liver, gall bladder and pancreas, and prostate cancer). The main increase in risk of fractures in this group was seen within the first year following diagnosis. A low risk group for fractures included all other cancer types (e.g. cancer of the breast, colon, skin etc). This may have implication for which patients should be selected for prevention against fractures.

Disclosures: ***P. Vestergaard****, None.*
This study received funding from: Danish Medical Research Council.

SA291

See Friday Plenary number F291.

SA292

Influence of Imatinib on Bone Remodeling in Juvenile Mice. J. Boehme*[1], M. Suttorp*[1], R. Fischer*[2], M. Bornhäuser*[3], J. A. Gasser[4]. [1]Department of Pediatrics, Division of Pediatric Hematology and Oncology, University Hospital Carl Gustav Carus, Dresden, Germany, [2]Institute of Pathology, University Hospital Carl Gustav Carus, Dresden, Germany, [3]Department of Internal Medicine I, University Hospital Carl Gustav Carus, Dresden, Germany, [4]Musculoskeletal Research, Novartis Institutes for BioMedical Research, Basel, Switzerland.

Imatinib (IM) is an effective treatment for chronic myeloid leukemia (CML) which inhibits the receptor tyrosine kinase BCR-ABL and other kinases like c-Kit, PDGF-R and c-FMS. The balance between bone resorption and bone formation under IM may be changed. We investigated the influence of IM on bone remodeling in growing mice.

Methods: At the age of 4 weeks, C3H mice were exposed to IM in drinking water for 10 weeks at doses of 80mg/kg/day (A), 110mg/kg/day (B), and 150mg/kg/day (C), resulting in serum levels of 60-674 ng/ml, 36-242 ng/ml and 51-534 ng/ml, respectively. Tibiae were analysed by pQCT, microCT and femora by histomorphometry. The plasma concentration of IM, osteocalcin, and the activity of the tartrate resistant acid phosphatase (TRAP5b) was determined.

Results: IM was tolerated well at all doses. At 5, but not 10 weeks, IM significantly and dose-dependently reduced the number of osteoclasts and resorption lacunae in femora of male animals from 23/field (controls) to 15 and 10, respectively. Effects were less pronounced in female mice.

Trabecular BMD was significantly increased in male mice by 8.5% and 9.9%, respectively, in groups B and C, and 5.2% in females (group C). Cortical thickness was increased in males by 6.1% and 11.2%, respectively, in group B and C and 7.5% in females (group C). Cancellous bone as assessed by microCT in males exhibited a significant increase in trabecular bone volume (BV/TV) of 19.7% and 13.2%, respectively, in groups B and C. In females a 10.6% increase was seen in group C. A significant increase in trabecular number (9.2% and 14.4%, respectively, in group B and C) in males and in females (12.8% in group C) was observed. As a result, trabecular connectivity improved significantly by 63% and 64%, respectively, in group B and C in males and by 22% and 38%, respectively, in group B and C in females. Bone biomarkers indicated a significant reduction of TRAP5b activity in groups B and C (both 5.1U/l) compared to controls (7.3U/l) and group A (6.5U/l) while osteocalcin levels remained unchanged.

Conclusion: In growing mice, IM reduced osteoclast number and resorption lacunae in long bones but not vertebrae. IM had a mild antiresorptive effect in cancellous bone (trabecular number), increased cortical thickness by inhibiting the expansion of the marrow cavity, and reduced TRAP5b activity. The effect was more pronounced in male mice and at younger age.

Disclosures: ***J.A. Gasser****, Employe of the Novartis Institutes for Biomedical Research 3.*

SA293

See Friday Plenary number F293.

SA294

TGF-beta Suppresses Adipocytic Differentiation and Enhances Accumulation of Stromal Cells in Myeloma Bone Lesions. K. Takeuchi*[1], M. Abe[1], M. Hiasa*[2], O. Tanaka*[1], S. Nakamura*[1], H. Miki*[1], K. Kagawa*[1], K. Yata*[1], T. Hashimoto*[1], S. Ozaki*[1], S. Kido[1], T. Matsumoto[1]. [1]Department of Medicine and Bioregulatory Sciences, University of Tokushima Graduate School of Health Biosciences, Tokushima, Japan, [2]Department of Orthodontics and Dentofacial Orthopedics, University of Tokushima Graduate School of Health Biosciences, Tokushima, Japan.

Multiple myeloma (MM) preferentially arises in the elderly, and develops devastating bone destruction. Although the bone marrow becomes fatty with normal aging, adipose tissue decreases and stromal cells increase in MM bone lesions. We have demonstrated that TGF-beta acts on stromal cells to inhibit their terminal differentiation into osteoblasts (OBs). TGF-beta is abundantly released and activated by enhanced bone resorption in MM bone lesions. Therefore, mesenchymal stem cell (MSC) differentiation into adipocytes may be suppressed and into stromal cells may be skewed, causing accumulation of stromal cells by enhanced TGF-beta actions in MM bone lesions. The present study was undertaken to clarify the role of bone-derived TGF-beta in MSC differentiation into adipocytes in MM. When C3H10T1/2 mesenchymal and ST2 stromal cells were cultured in an osteogenic medium with BMP-2, a considerable number of adipocytes appeared along with OBs. Addition of TGF-beta almost completely inhibited the adiopocytic differentiation, without affecting Runx2 expression. TGF-beta also suppressed adipocytic differentiation of C3H10T1/2 cells facilitated by a PPARgamma ligand, ciglitazone. To simulate MM bone lesions, we co-cultured rabbit bone marrow cells with MM cells on dentine slices placed on membrane filters, and examined ciglitazone-stimulated adiopocytic differentiation of C3H10T1/2 cells cultured in the lower chambers. Adiopocytic differentiation by ciglitazone was potently suppressed by TGF-beta, while blockade of TGF-beta actions by a type I receptor kinase inhibitor, SB431542, resumed adiopocytic differentiation. In co-cultures without dentine slices, C3H10T1/2 cells differentiated into adiopocytes. These results demonstrate that TGF-beta inhibits MSC differentiation into adipocytes while inhibiting terminal OB differentiation to create a stromal cell-rich microenvironment, and suggest that TGF-beta elaborated by enhanced bone resorption in MM bone lesions enhances accumulation of stromal cells to create “MM niche” suitable for MM expansion.

Disclosures: ***K. Takeuchi****, None.*

SA295

A New Automated Multiplex Assay for Simultaneous Measurements of 4 Serum Biochemical Markers of Bone Metabolism in Osteoporosis. A. Claudon*[1], P. Vergnaud*[1], C. Valverde*[1], A. Mayr*[2], U. Klause*[2], P. Garnero[3]. [1]Synarc, Biochemical Markers, Lyon, France, [2]Roche Diagnostics, Penzberg, Germany, [3]Synarc, Biochemical Markers / INSERM Research Unit 664, Lyon, France.

The serum biochemical markers C-terminal crosslinked telopeptides of type I collagen (CTX) for bone resorption, N-terminal propeptide of type I collagen (PINP) and osteocalcin (OC) for bone formation and intact PTH are commonly used in epidemiological and clinical trials in osteoporosis. Each of these markers is currently measured individually by manual or automated assays requiring a significant sample volume. A fully automated protein-array based assay was developed recently for the simultaneous measurement of these 4 markers in only 20 microliters of serum. The aim of this study was to evaluate the technical and clinical performances of this novel multiplex assay in osteoporosis.

Serum CTX, PINP, OC and PTH were measured by the multiplex (Imunological Multi Parameter Chip Technology, IMPACT, Roche Diagnostics) and the corresponding single automated reference assays (Elecsys, Roche) in 157 healthy premenopausal women, 74 healthy men and 56 postmenopausal osteoporotic women before and 6 months after treatment with oral ibandronate (150 mg/month).

Intra and inter assay variation of the multiplex assay was low and similar to single measurements (<10% for all markers). The lower limit of quantification (LLOQ) -which is the minimal concentration of endogenous marker that can be accurately and precisely determined- was lower for all 4 markers with the multiplex than with single assays, especially for CTX (0.023 vs 0.087 ng/ml). In premenopausal women, postmenopausal women with osteoporosis and healthy men, values determined by the multiplex technology highly correlated (r from 0.93 to 0.97, $p<0.0001$) with the corresponding single assays and absolute levels were comparable. After 6 months of treatment of osteoporotic women with monthly oral ibandronate, CTX, PINP and OC declined by a median of 48%, 63% and 52% ($p<0.0001$), decreases which were of similar magnitude as those observed with the corresponding single assays. Because of the improved sensitivity of the multiplex assay, CTX levels could be accurately and precisely determined in all samples after 6 months of ibandronate (all values above the LLOQ), whereas 28% of values were below the quantification limit of the single assay.

In conclusion, this novel fully automated protein-array assay allows the precise and sensitive measurements of serum CTX, PINP, OC and intact PTH simultaneously in a low serum volume. This assay should be useful for the investigation of bone metabolism in large clinical studies particularly when sample volume is limited.

Disclosures: ***P. Garnero****, None.*

SA296

Association of Urinary γ-Glutamyltransferase (GGT) and Serum FGF-23 with Prevalent Fracture: Hiroshima Cohort Study. S. Fujiwara[1], N. Masunari*[1], I. Takahashi*[1], W. Ohishi*[1], K. Ikeda[2]. [1]Clinical Studies, Radiation Effects Research Foundation, Hiroshima, Japan, [2]Department of Bone and Joint Disease, National Center for Geriatrics and Gerontology, Hiroshima, Japan.

We have reported that the urinary excretion of γ-glutamyltransferase (GGT), a recently identified bone-resorbing factor (JBC 2004, Endocrinology 2007), and the serum concentration of fibroblast growth factor (FGF)-23, an osteocyte-specific product, are associated with BMD (ASBMR 2007). The objective of the present study was to determine if urinary GGT and serum FGF-23 are associated with prevalent bone fracture in a population-based cohort. The study population consisted of 2,037 subjects (1,314 women and 723 men) aged 59 to 107 years old (average 73), followed up by biennial health examinations. A total of 252 subjects had spine fracture diagnosed by X-ray examination, and 125 had self-reported non-spine fracture. Serum FGF-23 was measured by ELISA. GGT level in the urine was determined by an autoanalyzer and corrected for creatinine concentration. BMD was measured in the spine and the hip by dual X-ray absorptiometry (QDR-4500, Hologic). Written informed consent was obtained from all participants. Multiple logistic regression was used for analysis.

Urinary GGT was significantly associated with prevalent spine fracture; when quartered (Q1-4), subjects with urinary GGT in the highest quartile (Q4) had a relative risk (RR) for spine fracture of 1.57 (95% confidence interval [CI], 1.05-2.34, p=0.03), after adjusting for age, sex and femoral neck BMD, compared with the Q2 reference group. Subjects with serum FGF-23 in the lowest quartile had RR for non-spine fracture of 1.96 (95% CI, 1.12-3.42, p=0.02). No relationship was found between GGT and non-spine fracture or between FGF-23 and spine fracture, after adjusting for age, sex and femoral neck BMD.

Thus, urinary GGT and serum FGF-23 are potential markers for prediction of fracture independently of BMD.

Disclosures: ***S. Fujiwara****, None.*

This study received funding from: National Institute of Biomedical Innovation.

SA297

See Friday Plenary number F297.

SA298

Comparison of the TRACP 5b Specificity of Two Commercial TRACP 5b Assays. H. Ylipahkala[1], K. M. Fagerlund[2], A. J. Janckila[3], J. M. Halleen[4]. [1]SBA Sciences Ltd, Oulu, Finland, [2]Anatomy, Institute of Biomedicine, University of Turku, Turku, Finland, [3]Medicine, Veterans Affairs Medical Center, Louisville, KY, USA, [4]Pharmatest Services Ltd, Turku, Finland.

Two forms of tartrate-resistant acid phosphatase (TRACP) circulate in human blood, TRACP 5a derived from macrophages and TRACP 5b derived from osteoclasts. Enzymatically active TRACP molecules constitute only 10% of circulating TRACP, while the remaining 90% circulates as fragments. We have studied the TRACP 5b specificity of commercially available BoneTRAP (IDS, Boldon, UK) and MetraTRAP5b (Quidel, San Diego, USA) assays and their clinical performance for monitoring alendronate treatment. BoneTRAP assay includes a monoclonal antibody O1A that binds both TRACP 5a and 5b, but not TRACP fragments. Bound TRACP activity is measured using pNPP as substrate at a TRACP 5b-selective pH 6.1. MetraTRAP5b assay includes two monoclonal antibodies, Trk49 that removes TRACP fragments and Trk62 that is stated to have high specificity for TRACP 5b. Bound TRACP activity is measured using CNPP as substrate at pH 6.4. Human serum TRACP 5a and 5b were separated by cation exchange chromatography and used to study TRACP 5b-specificity of the two assays. Both assays determined equal amounts of TRACP 5b activity, and the cross-reactivity to TRACP 5a was equal for both assays. When the substrate solutions were changed between the two assays, both assays still determined the same equal amounts of TRACP 5b activity as with their original substrates. Both assays determined approximately 5-fold less TRACP 5a activity with CNPP than with pNPP, indicating that CNPP is a more specific substrate for TRACP 5b. With pNPP as substrate, the antibody Trk62 bound TRACP 5a and 5b almost equally, indicating a very high cross-reactivity for TRACP 5a. The performance of the MetraTRAP5b assay is therefore mainly achieved by use of the more specific substrate CNPP, which partly compensates for the high cross-reactivity of the antibody Trk62 to TRACP 5a. We examined the clinical performance of both kits using a panel of serum samples from 137 postmenopausal women taking part in a placebo-controlled intervention trial, in which subjects received 5 mg alendronate per day. Alendronate treatment reduced TRACP 5b values by 53% when measured with the MetraTRAP5b assay, and by 42% when measured with the BoneTRAP assay. However, due to higher variability of the MetraTRAP5b assay, both assays showed similar sensitivity (80.0% vs 81.4%), signal-to-noise ratio (3.19 vs 3.14) and area under ROC curve (0.93 for both). We conclude that the BoneTRAP and MetraTRAP5b assays have equal specificity for TRACP 5b and interference by TRACP 5a, and equal clinical performance for monitoring alendronate treatment.

Disclosures: ***H. Ylipahkala****, SBA Sciences Ltd 2.*

SA299

PINP, a New Available Rat Bone Formation Marker. Usefulness in Osteopenia Studies Due to Androgen Lack and Ibandronate Treatment. M. Montero*[1], I. Quiroga*[2], M. Rubert*[1], M. Diaz-Curiel[3], F. Bauss*[4], C. De la Piedra[1]. [1]Biochemistry, Osteoarticular Pathology Laboratory, Fundacion Jimenez Diaz, Madrid, Spain, [2]Endocrinology, Hospital Puerta de Hierro, Madrid, Spain, [3]Internal Medicine, Fundacion Jimenez Diaz, Madrid, Spain, [4]Pharma Research Penzberg, Roche Diagnostics GmbH, Penzberg, Germany.

Ninety six Wistar rats, 9 months old, were sham operated (SHAM) or orchidectomized (OQX): untreated or treated with ibandronate (IBN) and were distributed into two studies, prevention (P) or treatment (T). P study: immediately after surgery animals were submitted to 4 groups which were administered subcutaneously for 20 weeks with either placebo (SHAM; n=12) and OQX (n=12) or with two regimens of IBN (Roche Diagnostics GmbH, Germany): 1 microg/Kg/day (OQX+dIBN; n=12) or 28 microg/kg/28 days (OQX+mIBN). T study: all animals were left untreated for 6 months after surgery and subsequently submitted to 4 groups treated in a similar way than in P study: (SHAM2; n=12), (OQX2; n=12), (OQX2+dIBN; n=12) and (OQX2+mIBN; n=12).After sacrifice, bone mineral density (BMD) was determined in the whole left femur by DEXA. Serum aminoterminal propeptide of collagen I (PINP, rat specific ELISA, IDS), and tartrate-resistant acid phosphatase, 5b isoenzyme (5b-TRAP ELISA, IDS) were measured. Five and 11 months after orchidectomy, OQX rats showed a decrease in PINP; 5 months after surgery OQX rats presented an increase in TRAP, that reverted 6 months later. Femoral BMD was decreased in OQX rats with respect to their SHAM groups. Both, daily or monthly treatment with IBN in P or T-studies, decreased TRAP and PINP indicating a general decrease in bone remodelling. Femoral BMD was “maintained like” or “recovered until” SHAM levels (P or T study respectively) in OQX groups treated with daily or monthly IBN. The above results suggest that PINP, a new available bone formation marker for rat experimental designs, is useful in studies about osteopenia due to androgen lack and remodelling effects of IBN treatment.

Disclosures: ***C. De la Piedra****, National Institute of Health (FIS PI 06/0025) 3; Hoffman La Roche 3.*

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SA300

Dickkopf-1 Predicts the Gain of Bone Mineral Density in Osteoporotic Women on Bisphosphonates. T. Kocjan*[1], G. Hawa*[2], B. Lindner*[2], S. Maitzen*[2], J. Prezelj*[1], Z. Trošt*[3], J. Marc*[3], S. Mencej*[3]. [1]Department of Endocrinology and Metabolic Diseases, Medical Centre Ljubljana, Ljubljana, Slovenia, [2]Research & Development, BIOMEDICA, Vienna, Austria, [3]Department of Clinical Biochemistry, Faculty of Pharmacy, Ljubljana, Slovenia.

Serum Dickkopf-1 protein (Dkk-1) is a potent inhibitor of bone formation. The aim of our study was to seek for correlation of serum Dkk-1 levels with change in bone mineral density (BMD) over a 1-year period in an osteoporotic female population treated with bisphosphonates.
51 postmenopausal women with newly diagnosed osteoporosis, who had been treated with weekly risedronate or monthly ibandronate plus calcium and vitamin D_3 supplements for 1 year were enrolled. The study was approved by the national medical ethics committee, and written informed consent was obtained from all participants.
Blood samples for determination of Dkk-1 and C-terminal cross linking telopeptide of type I collagen (CTX) were collected at the time of the diagnosis with first BMD measurement and after 3 months. Dkk-1 serum levels were determined by a newly developed ELISA-test. The relevance of circadian change in Dkk-1 serum levels was evaluated and found to be non significant. BMD measurement was then repeated after 1 year of treatment.
A significant negative correlation of serum Dkk-1 levels after 3 month was found with increase in spine and femoral neck BMD after 1 year of treatment.
This suggests that a single determination of serum Dkk-1 level after 3 months might be useful as an early predictor of long-term BMD gain determined by DXA after 1 year of osteoporosis treatment with bisphosphonates. Furthermore, high levels of serum Dkk-1 may identify postmenopausal women with osteoporosis who are not good candidates for bisphosphonate and might benefit more from anabolic treatment.
FUNDING: This work was supported by Grants Call CoOperate Enlarged Vienna 2005 provided by Zentrum für Innovation und Technologie, Austria and by Grants P3-0298 and P4-0127 provided by the Slovenian Research Agency.

Disclosures: ***G. Hawa****, None.*

SA301

See Friday Plenary number F301.

SA302

Development of a Bone Specific Alkaline Phosphatase Assay on the IDS Automated Analyser 3X3™. N. Baeyens*, A. K. Barnes*, G. Pirens*, G. Sarlet*, M. Bougoussa*, J. V. Leblon*, M. L. Garrity*. Immunodiagnostic Systems (IDS) Ltd, Bolton, United Kingdom.

Circulating levels of Bone Alkaline Phosphatase (BAP) are believed to reflect the metabolic status of osteoblasts which are involved in the formation of bone. Measurement of BAP has been shown to be useful in evaluating patients with Paget’s disease, osteomalacia, primary hyperparathyroidism, renal osteodystrophy, osteoporosis and metastases to bone.
BAP can be currently measured by the IDS OSTASE®* ELISA. The present work was conducted to transfer the manual assay onto the IDS Automated Analyser 3X3™. Multiple types of detection are embedded on this analyser, including spectrophotometric and chemiluminescent and will allow a rapid and robust transfer from a manual assay to a fully automated version, without any major modification in reagent formulation or loss of sensitivity. The proposed product will be a colorimetric assay using magnetic particles as the solid phase.
The assay uses the spectrophotometer present on board the analyser, reading the assay at 405 nm wavelength according to a kinetic method measurement. This kinetic value is directly proportional to the BAP activity. The protocol used in the analyser is directly derived from the IDS OSTASE® BAP kit and has been adapted to the analyser, with regard to the machine constraints (e.g. volume sample, time incubation, and washing process). In summary, 75 µl of sample are incubated for 15 min in the presence of antibody linked to biotin, magnetic particles coated to streptavidin are added and incubated for a further 15 min. The BAP/Antibody-biotin complex binds to the magnetic particles. These magnetic particles are captured with magnets and washed by the washing buffer used in the analyser. After that, the magnetic particles containing BAP fixed by antibody are incubated in the presence of pNPP and the absorbance is measured during a short time (300 sec) to obtain the kinetic constant in milli optical density / min at 405 nm.
The Bone specific Alkaline Phosphatase assay on the IDS Automated Analyser 3X3™ shows a measuring range of 2.5 µg/L to 120 µg/L with a analytical sensitivity ≤ 1µg/L, a functional sensitivity close to 2.5 µg/L and a inter-assay imprecision ≤ 7%. The assay demonstrates good correlation with IDS OSTASE® ELISA (from where the reagents are derived) with Analyser = 0.88 x ELISA + 0.25 µg/L with r = 0.91 (n=46).
These results indicate that the automated BAP assay can provide a sensitive and precise immunoassay with a good correlation with the IDS OSTASE® BAP ELISA kit.*Ostase® is a registered trademark of Hybritech Incorporated, a subsidiary of Beckman Coulter Inc.

Disclosures: ***N. Baeyens****, IDS Ltd 2.*

SA303

Development of a New N-Mid® Osteocalcin Immunoassay on the IDS Automated Analyser 3X3™. M. Bougoussa*, A. K. Barnes*, G. Pirens*, G. Sarlet*, N. Baeyens*, C. Hagelstein*, M. L. Garrity*. Immunodiagnostic Systems (IDS) Ltd, Bolton, United Kingdom.

Osteocalcin or bone Gla protein is a small 49aa protein that is rich in glutamic acid (GLA) and is a vitamin K-dependent protein that has been estimated to represent up to 20% of all non-collageneous protein in bone. A proportion of newly synthesized osteocalcin is released into the circulation, where it can be measured by immunoassay.
Assays for osteocalcin are not standardised, and different antibodies clearly recognize different fragments. Antibodies that recognise both the intact and the large N-terminal mid-molecule fragment appear to provide the best clinical information.
The IDS Automated Analyser 3X3™ N-Mid® osteocalcin assay is an adaptation of the IDS Nordic ELISA assay. This assay uses two monoclonal antibodies. The biotinylated antibody is directed against residues 21-29. The second antibody recognizes residues 10-16 and is coupled to an acridinium derivative. After an incubation of 50 µl of sample with the biotinylated and second antibody**,** 20 µl of streptavidin magnetic particles are added to the mixture. After a second incubation, followed by a washing step, triggers are added and the measured luminescence is directly proportional to the N-Mid® osteocalcin concentration present in the sample.
A first result is available after 45min. The analytical range of the assay is 2 to 120 ng/ml. The performance was established using the CLSI protocol. The analytical sensitivity is ≤0.5 ng/ml and the functional sensitivity is < 3ng/ml. The intra-assay and inter-assay precision are respectively < 6.5 % and <11 %. The osteocalcin recovery and linearity averages 106% and 102% respectively, expressed as Observed/Expected ratios. A correlation study was performed using 72 plasma and serum samples. The assay demonstrates good correlation with the IDS Nordic ELISA kit (R=0.97) and yields the following regression equation: IDS Automated Analyser 3X3™ = 0.92 x ELISA -2.73ng/ml.
The results indicate that the IDS Automated Analyser 3X3™ N- Mid® osteocalcin assay provides a sensitive, specific and reproducible assay with good correlation with the IDS Nordic ELISA kit.

Disclosures: ***M. Bougoussa****, IDS Ltd 2.*

SA304

Use of Conductivity for the Correction of Urinary N-telopeptide. S. R. Johnson, S. Carlilse*, A. Krishnankutty*, K. Higgs*, K. Zak*. R&D, SPD, Bedford, United Kingdom.

Urinary N-telopeptide (uNTx) measurements provide valuable clinical information on bone resorption and there are several assays available that can accurately measure uNTx. Measurements are normally corrected by dividing by the urine creatinine concentration to remove the influence of varying urine concentration. However, creatinine is an imperfect molecule for concentration correction because it is known to be affected by a number of variables such as muscularity, age and diet. Urine conductivity has previously been found suitable for normalising other urinary analyte concentrations, such as thromboxane B2, therefore its ability to correct uNTx measurements was examined. In particular, the influence of dietary sodium was studied as sodium ions are a major contributor of total urine conductivity.
An automated method for measurement of conductivity was developed, comprising of a biodot pump dispenser and X/Y/Z robotic arm with auto-sampler, in conjunction with a flow-through micro-conductivity cell and reader, which allowed high through put of samples. A model was built equating conductivity to creatinine using measurements from every single void of urine throughout a 24h period from 40 volunteers. This model was then applied to a study where volunteer's dietary sodium was controlled. Thirty volunteers were recruited and each spent 2 days at normal, low and high dietary sodium intake, during which they provided urine samples. Creatinine, conductivity and uNTx were measured on these samples to examine the robustness of the model.
The mean dietary sodium intake during low, normal and high sodium diets was 1.98, 3.24 and 14.89g for all volunteers. When conductivity measurements, converted using the non-linear model, were used to correct uNTx, mean concentrations for the low, normal and high dietary sodium intake were 29.27, 30.14 and 28.06 nM BCE/mM creatinine equivalent. Therefore conductivity was able to correct urine concentration under varying levels of sodium intake.
This analysis demonstrates that conductivity may be a viable alternative to creatinine for correction of uNTx. Conductivity measurements have the added benefit of being cheap, quick to perform and have low analytical variability.

Disclosures: ***S.R. Johnson****, None.*
This study received funding from: SPD

SA305

Usefulness of Alveolar Bone Density Measurement in Risk Assessment for Bisphosphonate-related Osteonecrosis of the Jaw (BRONJ). Y. Takaishi[1], A. Kamada[2], T. Ikeo[3], M. Nakajima*[4], T. Miki[5], T. Fujita[6]. [1]Takaishi Dental Clinic, Himeji, Japan, [2]Biochemistry, Osaka Dental University, Osaka, Japan, [3]Osaka Dental University, Osaka, Japan, [4]Oral and Maxillofacial Surgery, Osaka Dental University, Osaka, Japan, [5]Geriatric Medicine, Osaka City University, Osaka, Japan, [6]Internal Medicine, Katsuragi Hospital, Osaka, Japan.

Bisphosphonate-associated osteonecrosis of the jaw (BRONJ) is becoming a serious concern in dental and medical fields. Although risk factors such as intravenous long term use of potent bisphosphonates, dental surgical procedure including dental extraction and poor oral hygiene and infection have been pointed out, it is imperative to find a reliable test method predicting the occurrence of BRONJ. Around the lesions of BRONJ, increase of alveolar bone density is frequently noted. By using dental radiography with aluminum step wedge pasted to the film to standardize X-ray exposure, density of the alveolar bone was measured at several locations around the right mandibular premolar, placing the X-ray tube parallel to the film.
By using a software (Bone Right, Dentalgraphic•Com company, Himeji), data and histogram of the alveolar bone mineral density (al - BMD) were recorded on the screen of a lap - top computer in a few minutes. This method may also be applied similarly from a panorama film covering the whole series of the teeth in an individual. The radio-opaque BRONJ lesion is surrounded by relatively radiolucent area containing bacterial flora and inflammatory granulation tissue, presenting as chronic suppurative osteomyelitis. The bone mineral density around the osteonecrosis lesions including BRONJ showed an extremely high mineral density, 168±30 (SD) in 8 measurements in 4 cases of jaw osteonecrosis including 1 case following radiation treatment. Significantly higher than the values in 24 measurement in 6 case corresponding regions without BRONJ, 130±27, (P=0.0028). In one subject in whom two extractions were simultaneously carried out, BRONJ occurred only at the location with extremely high alveolar bone density, and not on other site of dental extraction with normal bone density. Alveolar BMD measurement thus appears to predict the occurrence of BRONJ to be useful for detecting subjects at systemic or local risk for occurrence of BRONJ, in addition to low serum CTX suggested as a systemic risk factor for BRONJ.

Disclosures: ***Y. Takaishi****, None.*

SA306

See Friday Plenary number F306.

SA307

Consistency in Measurement Assessments in Different Models of Norland DXA Scanners. T. V. Sanchez[1], D. K. Buckingham*[2], D. R. Purvis*[2], C. A. Dudzek*[2]. [1]Research and Development, Norland--a CooperSurgical Company, Socorro, NM, USA, [2]Research and Development, Norland--a CooperSurgical Company, Fort Atkinson, WI, USA.

The consistency in the measurements made by different models of equipment is an issue in the densitometry community. To examine consistency in how Norland densitometers evaluate bone mineral density, bone mineral content and bone area we have examine scanner measurements done between 1988 and 2008 in processing measurements done on one of two AP Spine phantoms.
As part of the normal production process, at final testing Norland scanners carry out nine scans on one of two AP Spine Phantoms using standard AP Spine scan settings for the model and software being used. To examine consistency in measurements we randomly selected records from twenty-five different scanners of each model produced between January of 1988 and March of 2008. Models represented in this collection included the Norland XR-26, Eclipse, XR-36, Excell, XR-46, XR-600 and XR-800 systems. Each scanner used software current to its production date. The resulting average and standard deviation were compared for each model.
Results (%True) of Measurements on the Primary AP Spine Phantom by Different Scanner Systems Between 1988 and 2008.

	BMD	BMC	Area
XR-26	100.15±0.54	99.89±0.53	99.72±0.35
Eclipse	99.71±0.71	99.49±0.73	99.80±0.44
XR-36	99.95±0.49	99.66±0.54	99.69±0.29
Excell	100.63±0.44	101.22±0.52	100.59±0.38
XR-46	100.50±0.78	100.33±1.01	99.96±0.53
XR-600	100.12±0.24	100.68±0.41	100.56±0.34
XR-800	100.17±0.42	100.33±0.60	100.16±0.43

The results show a consistency in measured bone mineral density, bone mineral content and bone area in the various scanners. The results also demonstrate how the use of the same phantom over time can be employed to demonstrate that equipment is performing consistently over changes in hardware or software.

Disclosures: ***T.V. Sanchez****, None.*

SA308

See Friday Plenary number F308.

SA309

The Effect of Vertebral Marrow Fat Content on the Diagnosis of Osteoporosis. G. M. Blake[1], J. F. Griffith*[2], D. K. W. Yeung*[2], P. C. Leung*[2], I. Fogelman[1]. [1]King's College London School of Medicine, London, United Kingdom, [2]Prince of Wales Hospital, Chinese University of Hong Kong, Shatin, Hong Kong.

Quantitative examination of iliac crest bone biopsies shows that as subjects become older bone and functional marrow are replaced by adipose tissue. Studies of vertebral marrow fat using nuclear magnetic resonance spectroscopy (MRS) show a highly significant relationship between spine T-score and marrow fat content. These findings suggest that the ability of dual energy x-ray absorptiometry (DXA) scans to identify patients at high risk of fracture is partly explained by the negative effect of increasing marrow fat on bone mineral density (BMD). However, care is necessary in interpreting the relationship between spine T-score and vertebral marrow fat because of a selection effect in which subjects with higher marrow fat are more likely to be found to have osteoporosis.
We studied groups of elderly Hong Kong Chinese women (N = 103, mean age 73 y; range 67-84 y) and men (N = 82, mean age 73 y; range 67-101 y) who had spine DXA scans and MRS measurements of L3 marrow fat. The effect of varying marrow fat on BMD was modelled using vertebral body thicknesses measured in 50 men and women. Spine T-scores in each individual were adjusted for the measured marrow fat. Subjects were sorted into their WHO categories based on corrected T-scores, and the relationship between marrow fat and T-score status evaluated using regression analysis and analysis of variance. The average change in marrow fat per T-score unit was used to infer the proportion of the spine BMD fracture discrimination explained by marrow composition.
The mean (SD) of the L1-L4 vertebral body thickness was 30.2 (2.1) mm for the Hong Kong women and 33.4 (2.5) mm for the men. When the marrow fat content measured by MRS changed from 0 to 100% the BMD change was estimated to be 0.14 g/cm^2 (1.3 T-score units) in women and 0.16 g/cm^2 (1.3 T-score units) in men. Adjustment of spine BMD for marrow fat reduced the significance of the correlation between marrow fat and T-score in both men and women compared with uncorrected data. However, there was still a trend for marrow fat to increase with decreasing T-score with a slope of -1.2 ± 0.7% per T-score unit ($p = 0.078$) for women and -1.4 ± 0.6% per T-score unit ($p = 0.023$) for men. When the effect of marrow composition on fracture discrimination was evaluated the results showed that 2% of the ability of spine DXA measurements to discriminate fracture risk is explained by the higher vertebral marrow fat content found in osteoporotic subjects. In conclusion, after adjustment for the selection effect patients with osteoporosis were still found to have higher vertebral marrow fat content. However, marrow composition made a negligible contribution to fracture discrimination.

***Disclosures:** **G.M. Blake**, None.*

SA310

See Friday Plenary number F310.

SA311

Correlations between Panoramic Radiomorphometric Indices and Bone Mineral Density in Postmenopausal Women. A. F. Leite*[1], P. T. S. Figueiredo*[2], C. M. Guia*[3], A. P. Paula[4], N. S. Melo*[5]. [1]Oral Radiology, Department of Dentistry, College of Health Sciences, University of Brasília, Brasilia, Brazil, [2]Oral Radiology, Department of Dentistry, College of Health Sciences, University of Brasília, Brasília, Brazil, [3]College of Health Sciences, University of Brasília, Brasília, Brazil, [4]Rheumatology Division, College of Health Sciences, University of Brasília, Brasília, Brazil, [5]Department of Dentistry, College of Health Sciences, University of Brasília, Brasília, Brazil.

The aims of this study were to correlate seven panoramic radiomorphometric indices with the BMDs of L1-L4, femoral neck and total hip, and to evaluate the accuracy of these indices in predicting densitometric diagnoses of osteoporosis, and T-Scores ≤ -2.0. Three hundred and fifty one healthy postmenopausal women aged over 45 years were selected. All performed dual X-ray absorptiometries (QDR 1000, Hologic, USA) and panoramic radiographs (Rotograph Plus; Villa Medical System, Italy). Mandibular cortical indices, simple visual estimations of cortical widths, mental and antegonial indices, antegonial widths, and gonial and antegonial angles were measured. A stepwise forward logistic regression adjusted for age was performed. Significant associations were demonstrated between qualitative indices, cortical measurements and BMDs at the three bone sites ($p < 0.001$). In women with severely eroded mandibular cortices, classified as C3 by the mandibular cortical index, the odds ratio (OR) was 4.82 for having osteoporosis at one of measured sites, and 10.87 for a T-Score ≤ -2.0. In women with very thin mandibular cortices, determined by simple visual estimation of cortical width, the ORs were 8.02 and 5.46 for osteoporosis and T-Score ≤ -2.0, respectively. In conclusion, certain qualitative indices, simpler and easier to be applied, may be considered more accurate panoramic measurements in predicting osteoporosis and T-Score ≤ -2.0.

***Disclosures:** **A.F. Leite**, None.*

SA312

See Friday Plenary number F312.

SA313

6819 Analysis on Measurement of Bone Mineral Density of Phalanges by Radiographic Absorotiometry in Beijing. J. Wang*[1], Z. Liu[2], Z. Zhang*[1], P. Zhong*[1], Q. Wang*[1], X. BI*[3], L. Al-Dayeh*[3]. [1]Aviation Industry Center Hospital, Beijing, China, [2]China Osteoporosis Fundation, Beijing, China, [3]CompuMed, Inc., Los Angeles, CA, USA.

Osteoporosis is a very common disease in aging people in China. According to China's 2000 national census, approximately 100 million of its citizens suffer from various stages of osteoporosis. Although the T-Score based WHO criteria for osteoporosis diagnosis were derived from Bone Mineral Density (BMD) measurement of Caucasian women, they are still the mostly referenced standards in practice in China. It is very important to establish Chinese diagnostic standards for osteoporosis prevention and diagnosing.
Objective: To measure phalangeal BMD of normal population in Beijing area using Radiographic Absorptiometry(RA); to establish Chinese normal reference database for RA technique and to compare BMD measurement results by RA with that of forearm by SPA. Methods: A study group consisted of 6819 healthy participants aged between 10 to 90 years old (with male 3376, female 3443) was investigated. Middle phalangeal BMD of each participant's index, middle and ring fingers in the non-dominant hand were measured using RA technique (OsteoGram2000 CompuMed, Inc, U.S.A.). The measured results were calculated and grouped according to an age interval of every 10 years. Results: The bone status of each participant was evaluated by two commonly used standards in China; which are the WHO and OCCGS. According to the WHO standard 21% of the females and 4% of the males among the investigated people had osteoporosis status. With the OCCGS standard 33% of the females and 10% of the males had osteoporosis status. Conclusion 1. A normal reference database for phalangeal BMD by RA technique has been established in China for the first time. 2. The OCCGS standard with a cutting point at -2.0SD might be more appropriate than that of the WHO standard of T-score =-2.5 for Chinese population in osteoporosis disease screening and evaluation when BMD test was performed using RA. 3. The BMD results measured by RA technique are highly correlated to that of forearm by SPA in China.

***Disclosures:** **X. Bi**, None.*

SA314

Feasibility and Reproducibility of *in-vivo* Assessment of Trabecular Bone Architecture using MicroMRI-based Method at Multiple Study Centers. N. B. Watts[1], S. L. Greenspan[2], R. Jackson[3], M. Maricic*[4], W. Liu*[5], B. Kuzmak[6], A. Grauer*[6], K. Driver*[7], T. Dufresne*[7], P. Chmielewski*[7], B. R. Gomberg*[8], P. Seaman*[8], B. Borah[7]. [1]University of Cincinnati, Cincinnati, OH, USA, [2]University of Pittsburg, Pittsburg, PA, USA, [3]Ohio State University, Columbus, OH, USA, [4]Catalina Pointe Clinical Research, Tucson, AZ, USA, [5]UMDNJ, Newark, NJ, USA, [6]P & G Pharmaceuticals, Mason, OH, USA, [7]P&G Pharmaceuticals, Mason, OH, USA, [8]MicroMRI Inc., Philadelphia, PA, USA.

In-vivo imaging methods such as microMRI and peripheral quantitative CT (pQCT) allows evaluation of architectural changes in common peripheral skeletal sites that commonly fracture. The microMRI method for bone architecture measurement has been validated in a research setting. Prior to use in a longitudinal clinical study, the feasibility of the method needed further evaluation under realistic multicenter study conditions. We assessed the repeatability and reproducibility of architectural indices of the distal radius of the same 6 premenopausal females (age 29 - 42 years) at 5 study centers using a custom-made wrist coil on 1.5 Tesla GE MRI scanners. At each study center, each subject had 2 scans/day on 2 consecutive days performed by the same operator. At 1 center, 2 additional scans were obtained for each subject by a different operator. Data from 1 center was also analyzed by 2 analysts. All scans were completed within about 2 months. Bone volume fraction (BV/TV) and topological parameters (eg, surface to curve ratio and erosion index) were analyzed by MicroMRI Inc. using proprietary software. Additional conventional parameters of trabecular architecture were generated from the raw data using software package developed by Scanco Medical, Inc., available on microCT scanners. The reproducibility was assessed by root mean square coefficient of variations (RMS-CVs) and intraclass correlation coefficients (ICCs) of each of the parameters. Based on the within-site, motion-corrected, slice-matched data, the RMS-CVs for BV/TV and topological parameters ranged from 2.7 - 5.1% and 6.6 - 11.8%, respectively, and are comparable to historical data. The ICCs (≤ 0.75) found in this study were lower than historical data (>0.80) and may be due to small variance in structural parameters observed in the homogeneous female subjects in this study. BV/TV generated by microMRI and Scanco microCT methods compared favorably with minor differences in RMS-CV and ICC. The operator-to-operator and analyst-to-analyst variability was negligible. In conclusion, results of this study showed that the microMRI method was reproducible and repeatable at multiple study sites with multiple operators and has potential to be used in longitudinal clinical studies to assess treatment-modified changes in bone architecture.

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This study received funding from: The Alliance for Better Bone Health.

SA315

See Friday Plenary number F315.

SA316

Usefulness of SpinalMouse as a Screening Tool for the Presence of Vertebral Wedge Deformity. K. Mikawa, Y. Abe, K. Aoyagi. Public Health, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan.

The purpose of this study was to evaluate the ability of a novel clinical tool, SpinalMouse®, to identify women with vertebral wedge deformities. Subjects comprised 102 women with a mean (±standard deviation) age of 77.5 ±6.7 years (range, 62-97 years) who visited an orthopedic outpatient clinic due to lower back pain. We measured curvature of the thoracic and lumbar spine using SpinalMouse®, and thoracic kyphotic angle and lumbar lordotic angle were calculated. Lateral spine radiography was performed and radiographic vertebral wedge deformities were assessed by quantitative morphometry. Vertebral anterior and posterior heights were measured and anterior/posterior (AP) ratio was calculated. Vertebral wedge deformity was considered present for AP ratio <0.8. Other types of vertebral deformity (i.e., central deformity and crush deformity) were not included in this study. Of the 102 subjects, 48 showed ≥1 thoracic vertebral wedge deformity and 39 had ≥1 lumbar vertebral wedge deformities. Women with thoracic vertebral wedge deformities displayed a significantly greater thoracic kyphotic angle than women without any thoracic vertebral wedge deformity. Similarly, women with lumbar vertebral wedge deformities showed significantly smaller lumbar lordotic angle than women without any lumbar vertebral wedge deformity. Receiver operating characteristic analysis showed that both thoracic kyphotic angle and lumbar lordotic angle were useful in discriminating women with wedge deformities in respective lesions. Area under the curve was 0.95 for thoracic kyphotic angle in discriminating women with thoracic vertebral wedge deformities and 0.85 for lumbar lordotic angle in discriminating women with lumbar vertebral wedge deformities. When a thoracic kyphotic angle of 47° was used as a cutoff, specificity and sensitivity for identifying individuals with thoracic vertebral wedge deformities were 92% and 91%, respectively. As for lumbar spine, when a lumbar lordotic angle of 3° was used as a cutoff, specificity and sensitivity for identifying individuals with lumbar vertebral wedge deformities were 87% and 75%, respectively. These results suggest that increased thoracic kyphosis and decreased lumbar lordosis as assessed by SpinalMouse® are significantly associated with existing vertebral wedge deformities in the respective lesions. SpinalMouse® could be useful as a screening tool to identify individuals requiring radiographic assessment for vertebral wedge deformities.

***Disclosures:** **K. Mikawa**, None.*

SA317

See Friday Plenary number F317.

SA318

Collecting Bone out of the Medial Condyle of Tibia - A New Method. S. Jirsakova*[1], V. Vyskocil[2], A. Nemeckova*[3], R. Pikner*[4], J. Michalek*[5]. [1]Department of Bone disease, Charles University Hospital, Plzen, Czech Republic, [2]Metabolic Bone Disease Center, Charsles University Hospita, Plzen, Czech Republic, [3]Department of Histology and Embryology, Charles University, Plzen, Czech Republic, [4]Metabolic Bone Disease Center, Charles University Hospital, Plzen, Czech Republic, [5]Academy of Sciences of The Czech Republic, Prague, Czech Republic.

The aim of our study was to design and develop a new osseous tissue specimen collecting method that would show advantages in comparison with methods already used.
We evaluated microcracks, dynamic parameters and histomorphometry on bone biopsies from medial condyle of tibia in 35 postmenopausal women (age 60-80 yr) who had received bisphopshonate therapy for at least 9 years. We compared results with bone biopsies obtained from 10 cadavers (age 65-78 yr). Bone biopsies from medial condyle of the tibia were obtained after tetracycline double-labeling. The biopsies were taken with a mechanical bone trephine. Bone samples were bulk stained with green calcein as second flourochrome. The microcracks and dynamic parameters were evaluated with the help of a laser confocal scanning microscope. The histomorphometry analysis of the bone samples was evaluated from semi-thick sections under light microscope.
In all alendronate-treated patients the newly formed bone retains its lamellar structure, erosion cavities are shallow, osteoclasts only have few nuclei and there was no evidence of marrow fibrosis or cellular toxicity. Six women treated long-term-wise with alendronate have no visible microfractures. Among treated women, cancelous bone microcrack frequency of bone tissue was with the median crack density 0,130 microcracks/mm^2, which did differ significantly from that observed in untreated group (0,085 microcracks/mm^2). The mean cancellous bone microcrack length was 160 µm among treated women and did differ significantly from that observed in untreated group (80 µm).
The mean MAR was normal in treated patients; 0.71 ± 0.16 µm/d. Bone remodeling was suppressed in the treated group, with the mean Ac.f. = 0.08 ± 0.07 /year, MS/BS = 0.625 ± 0.713%, BFR/BS = 0.005 ± 0.004 µm^3/µm^2/day.
The authors developed and described a method for collecting and evaluating bone specimens of osseous tissue from the median condyle of the tibia. Bone specimens collected by the recommended method met the parameters reguired for qualitative, quantitative histomorphometrics analysis and microfracture analysis. This method shows advantages in clinical practice in comparison with methods already used.

***Disclosures:** **V. Vyskocil**, None.*

SA319

See Friday Plenary number F319.

SA320

Morphometric Determinants of Three Dimensional Femoral Neck Structure and Strength in Older Postmenopausal Women. R. L. Prince[1], K. Zhu[2], M. Pollock*[1], V. H. S. Low*[3]. [1]School of Medicine and Pharmacology, University of Western Australia, Perth, Australia, [2]Department of Endocrinology and Diabetes, Sir Charles Gairdner Hospital, Perth, Australia, [3]Department of Radiology, Sir Charles Gairdner Hospital, Perth, Australia.

Both height and weight are determinants of areal BMD. However the relative importance of body mass and stature to true three dimensional bone structure and strength remains uncertain. This study examines the relationship between body mass and size to femoral neck structure measured using quantitative computed tomography (QCT) and the bone strength calculated from engineering principles.
The study subjects were 186 women aged 73.6 ± 8.2 years enrolled for a dietary intervention study. Potential physical determinants were height, weight and calf girth. Bone structure was measured by QCT (Phillips Brillance CT) with patients lying on top of the QCT Pro™ calibration phantom and analysed with Mindways QCT Pro software. Linear regression analysis of the determinants of femoral neck structure and strength variables was undertaken using the independent variables age, height, weight and calf girth.
Both height and weight accounted for some of the variance of areal femoral neck (FN) BMD (R^2 Height 5.8%, Weight 5.8%, $P < 0.01$) but not true volumetric FN BMD although there were strong independent correlations of height and weight with FN mass (R^2 Height 15.1%, Weight 8.9%, $P < 0.01$) and volume (R^2 Height 13.4%, Weight 6.7%, $P < 0.01$).
In multiple regression the only significant predictor of total and trabecular FN cross-sectional bone area, related to bone strength in compression, was height accounting for 16% and 7.4% of variation respectively. Regarding femoral neck section modulus, a measure of femoral neck strength in bending, both in plane and out of plane measures were related to height at 19.7% and 16.3% of variation respectively.
These results show that in elderly postmenopausal women true three dimensional volumetric FN BMD is not related to body size. This is because although both FN mass and volume are body size dependent this dependency is proportional and cancels out in the calculation of true BMD. However FN bone strength in compression and vending is related to stature presumably because of the bone size dependency of these measurements. Thus although the relationship between body size and areal FN BMD is an aberration of the method the fact that size is a predictor of strength may account for the clinical predicative value of areal bone density combining as it does both bone size and bone mass into one value.

***Disclosures:** **R.L. Prince**, None.*
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SA321

See Friday Plenary number F321.

SA322

Validation of a 2D/3D Generic Mathematical Relationship Between TBS as Assessed by DXA, and BV/TV and TbTh as Assessed by Micro Computed Tomography: An Experimental Study Based on Human Cadaver Vertebrae. L. Pothuaud[1], N. Barthe*[2], M. Isidore*[3], P. Carceller*[1], D. Hans[4]. [1]PTIB - University Hospital of Bordeaux, Med-Imaps, Pessac Cedex, France, [2]University Hospital of Bordeaux, Biophysics Laboratory, Bordeaux, France, [3]University Hospital of Bordeaux, Department of Nuclear Medicine, Bordeaux, France, [4]Center of Bone Disease, Department of Bone and Joint Disease, Lausanne University Hospital, Switzerland.

In a previous study [1], we have established a significant mathematical relationship between Trabecular Bone Score (TBS) as evaluated on 2D simulated projection image and 3D characteristics of bone microarchitecture: bone volume fraction (BV/TV) and trabecular thickness (TbTh). The aim of this study was to independently evaluate the accuracy of this mathematical relationship based on DXA examination of human cadaver vertebrae.
20 dried human cadaver vertebrae were measured on an iDXA densitometer (GE-Lunar) with a specific positioning system miming standard antero-posterior acquisition and immerged in 17 cm of water. A region of interest (ROI) was defined on DXA scan. The DXA image was then exported on a specific workstation for TBS calculation. 3D reconstructions of the vertebrae were obtained by µCT (eXplore Locus, GE). The calibrated 3D grey-level images were analyzed using MicroView-GE software with specific add-on. Auto-threshold was applied and 3D-ROI was defined enclosing complete bone microarchitecture. Standard basic parameters, BV/TV and TbTh, were evaluated in this 3D-ROI. The fit-coefficients $\{a_{00}, a_{01}, a_{10}, a_{11}\}$ of the generic mathematical relationship TBS = $[a_{00}+a_{01}*Ln(TbTh)] + [a_{10}+a_{11}*Ln(TbTh)]*BV/TV$ were determined following non-linear regression analysis. The accuracy of the 2D/3D mathematical relationship was evaluated by correlation coefficient and root mean square error between estimated (indirectly from µCT) and experimentally (from DXA image based on in house algorithm [1]) measured values of TBS. The fit-coefficients were: a_{00}=-0.717, a_{01}=-0.510 (p=0.0043), a_{10}=0 (not considered coefficient), and a_{11}=-2.028 (p<0.0001). TBS value estimation was expressed as TBS = -0.717 - 0.510*Ln(TbTh) - 2.028*Ln(TbTh)*BV/TV, and led to a relative accuracy characterized by r = 0.93 and RMS-SD=0.0487.
In our study we have confirmed the accuracy of a generic mathematical 2D/3D relationship from DXA examination of human cadaver vertebrae. TBS as evaluated from DXA image directly represents a score of two 3D characteristics of bone microarchitecture BV/TV and TbTh.
[1] Pothuaud L., Carceller P., Hans D. Correlations between grey-level variations in 2D projection images (TBS) and 3D microarchitecture: Applications in the study of human trabecular bone microarchitecture. Bone 2008 Apr;42(4):775-87.

Disclosures: ***L. Pothuaud****, None.*

SA323

See Friday Plenary number F323.

SA324

To Avoid Underdiagnosis of Vertebral Fracture, Recognition of True Fracture Line Including Multiple Schmorl's Node Is Necessary. S. Okamoto[1], H. Noguchi*[2], A. Itabashi[3], H. Suzuki*[4], S. Okamoto[5]. [1]SORF Okamoto Clinic, Oita, Japan, [2]Noguchi Thyroid Clinic and Hospital Foundation, Beppu, Japan, [3]Saitama Center of Bone Research, Saitama, Japan, [4]Suzuki Orthodontic office, Nagasaki, Japan, [5]KS Okamoto Clinic, Shimabara, Japan.

Comparative studies between TV-X ray fluoroscopy with the 3-dimensional CT are performed. The 3-D models showed the vertebral fracture lines are rarely smooth but consist of a mixture of perforated indentations or Schmorl's nodes. To avoid underdiagnosis of vertebral fracture, recognition of true fracture line is definitely necessary. TV-X ray fluoroscopy was performed in 136 normal volunteers and 2,740 female osteoporosis patients. Also we created computerized 3-D renderings of 1280 cases. To make the X-ray pass through straight on the vertebral bodies, we asked the patients in an upright position to move their positions so that the distal and proximal edges of each vertebra would coincide. In 37 young female volunteers, the mean C/P ratios coincide with reported autopsy data of young females who died in their 20s or 30s. The mean is about 15% lower than that of reported conventional X-ray data. The widely used SQE standard stands on the image that a C/P ratio of a mild deformity, 80-75%. In fact, normal vertebrae have -15 to -20% concavity. That is, the vertebral terminal plates are not flat. For example, mean C/P ratio of L2 vertebra is 83% by the analysis of autopsy, CT or TV fluoroscopy, while that of plain X-ray assessment is 98%. That is, conventional X-ray analysis by mid-point digitization made as much as 15% error in measurement. The rim lines of vertebra almost always have remained intact and straight. These lines are readily seen on the plain X-ray. This "flat terminal plate" misconception leads to the serious misdiagnosis of vertebral fractures. The VFA method sometimes uses the rim line to measure and at other times the fractures line, caused by the vagueness of the image. As few osteoporotic fractures are symmetric, blindly taken radiographs or VFA can not avoid obliquely tilted or horizontally rotated vertebral figure. Even though the patient is going to lie down straight, vertebral bodies are tilted. Without positional adjustment, fracture lines are often mistaken for vertebral rim lines in the assessment. For osteoporotic fracture detection, only C/P ratio measurement is enough by our method. Our practical C/P standard ratios (YAM mean -2.5 S.D.) apparent for fractured vertebra were lower than 0.67 at T4 to T7 level, 0.69 at T8 to T11, 0.71 at T12 to L3 or 0.74 at L4 level.3D CT also can detect osteoporotic vertebral fractures which are often overlooked behind spinal OA. These finding suggests that vertebral fractures are included in the group of "disk herniation" into the weakened vertebral body through the perforated endplates.

Disclosures: ***S. Okamoto****, None.*

SA325

Effect of Parity on Bone Mineral Density in Premenopausal Women: JPOS Cohort Study. J. Tamaki[1], M. Iki[1], A. Morita*[2], Y. Sato*[3], E. Kajita*[4], S. Kagamimori*[5], Y. Kagawa*[6], H. Yoneshima*[7]. [1]Public Health, Kinki Univ. School of Med., Osaka-Sayama, Japan, [2]Nutritional Education Program, National Institute of Health and Nutrition, Tokyo, Japan, [3]Domestic Sciences, Jin-ai Women's College, Fukui, Japan, [4]Public Health & Home Nursing, Nagoya Univ. School of Health Sciences, Nagoya, Japan, [5]Welfare Promotion & Epidemiology, Univ. of Toyama, Toyama, Japan, [6]Kagawa Nutrition Univ., Tokyo, Japan, [7]Shuuwa General Hospital, Kasukabe, Japan.

Only cross-sectional observational or case-control studies have been available which evaluate the effect of parity on BMD. More than 15% of Japanese women between the ages of 20 and 40 are categorized in leanness, and the proportion of young women with less than standard BMI (22kg/m^2) would be relatively large across Asia. Therefore, to clarify the effect between parity and body weight on BMD in women at peak bone mass, we analyzed a representative sample of Japanese women with the Japanese Population-based Osteoporosis (JPOS) Cohort Study .
We conducted a baseline survey in 1996 and follow-up surveys in 1999 and 2002. We analyzed 654 premenopausal women aged 20-44 years in 1996 cross-sectionally, and 253 women younger than 45 in 2002 longitudinally. BMD at the lumbar spine (LS), total hip (TH), and the distal 1/3 radius (DR) was examined. For sub-analyses, subjects with BMI less than 24.2 kg/m^2 were analyzed cross-sectionally and longitudinally.
To clarify the effect of parity after complete recovery from delivery on BMD, multiple liner regression analysis of BMD in 1999 among 224 subjects aged less than 45 years with BMI <24.2 without delivery between baseline and 3 year follow-up was conducted, which revealed one parity was significantly associated with increased BMD at LS or DR after adjusting for age, weight, height, calcium intake, smoking habit, and exercise habit.
As results of multiple liner regression analysis predicting for change of BMD between baseline and 6 year follow-up among 160 subjects aged less than 45 years with BMI <24.2, delivery between the first 3 years was significantly associated with increased BMD at LS, one parity at baseline was significantly associated with increased BMD at TH or DR, and delivery between the first 3 years was significantly and negatively associated with BMD at DR, in each model including age, weight, height, BMD in 1996, change of body weight in 6 years, parity in 1996, delivery during the first 3 years, and delivery during the last 3 years as dependent variables. The analysis among total 654 subjects revealed no significant effect of parity.
Our findings would suggest a positive relationship between parity and bone density at lumbar spine among premenopausal women with standard BMI or less.

Disclosures: ***J. Tamaki****, Japanese Society for the Promotion of Science 5.*
This study received funding from: the Japan Milk Promotion Board and the Japan Dairy Coucil, the Japanese Society for the Promotion of Science, the Research Society for the Metabolic Bone Diseases.

SA326

Reimbursement for Bone Mineral Density Testing Among U.S. Medicare Beneficiaries. J. R. Curtis[1], A. Laster*[2], D. J. Becker*[3], L. Carbone[4], M. Kilgore*[3], R. Matthews*[5], M. A. Morrisey*[3], K. G. Saag[1], S. B. Tanner[6], E. Delzell*[5]. [1]Division of Rheumatology, University of Alabama at Birmingham, Birmingham, AL, USA, [2]Arthritis & Osteoporosis Consultants of the Carolinas, Charlotte, NC, USA, [3]Department of Health Care Organization and Policy, University of Alabama at Birmingham, Birmingham, AL, USA, [4]Veterans Administration Medical Center, University of Tennessee, Memphis, TN, USA, [5]Department of Epidemiology, University of Alabama at Birmingham, Birmingham, AL, USA, [6]Department of Medicine, Vanderbilt University, Nashville, TN, USA.

Introduction: Although the Bone Mass Measurement Act outlines the indications for central dual energy x-ray absorptiometry (DXA) testing for U.S. Medicare beneficiaries, the practical considerations regarding reimbursement are sometimes ambiguous. We evaluated the impact of gender, ICD-9 code submitted, time since previous DXA, and local Medicare carrier on whether or not the claim was reimbursed.
Methods: Using Medicare data from 1999-2005, we studied beneficiaries age ≥ 65 with part A+B, not HMO enrollees and in the 5% sample. We identified central DXA claims and evaluated the relationship between reimbursement for DXA and gender, ICD-9 code submitted for reimbursement of central DXA (CPT code 76075), interval of time since a preceding DXA, and Medicare carrier. Multivariable logistic regression was used to evaluate the independent relationship between carrier and reimbursement for DXA.
Results: For persons that had no DXA in 1999 or 2000 and who had one in 2001 or 2002, the percentage of DXAs claims denied was 5.3% for women and 9.1% for men. Rates of denial for repeat DXAs performed within 23 months was approximately 19% and did not differ by gender. There was substantial variability in reimbursement by ICD-9 diagnosis code submitted and varied by more than 6-fold. For DXAs repeated at < 23 months, the proportion of claims denied ranged from 2% to 43%, depending on the Medicare carrier.
Conclusion: Reimbursement for DXA varies significantly by gender, time since previous

DXA, ICD-9 diagnosis code submitted and local Medicare carrier. Greater guidance and transparency in coding policies is needed to improve access to DXA as a covered service for persons with Medicare.

Number of DXAs Performed and Proportion Not Paid, by Gender and Testing Interval

	Women		Men		
	Number Performed	Proportion Not Paid (%)	Number Performed	Proportion Not Paid (%)	P value*
Initial DXA	72700	5.3	6760	9.1	< 0.0001
Repeat DXA< 23 months	7749	18.5	826	19.0	0.75
Repeat DXA≥ 23 months	26687	3.3	1548	4.8	0.002

Disclosures: ***J.R. Curtis****, Novartis, Amgen, Merck, Proctor & Gamble, Eli Lilly, Roche 3; Roche, UCB, Proctor & Gamble 2; Merck, Procter and Gamble, Eli Lilly, Novartis, Roche 1.*

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SA327

See Friday Plenary number F327.

SA328

Poor Peripheral Nerve Function Is Related to Lower BMD: The Osteoporotic Fractures in Men Study. E. S. Strotmeyer[1], K. A. Faulkner[1], A. D. Iuliano*[1], J. M. Zmuda[1], A. V. Schwartz[2], M. Petit[3], E. Orwoll[4], J. A. Cauley[1]. [1]Epidemiology, University of Pittsburgh, Pittsburgh, PA, USA, [2]University California, San Francisco, San Francisco, CA, USA, [3]University of Minnesota, Minneapolis, MN, USA, [4]Oregon Health & Science University, Portland, OR, USA.

Bone tissue is innervated and neurotransmitters directly affect bone remodeling. Poor motor peripheral nerve (PN) conduction was related to lower hip bone mineral density (BMD) in older adults in a dose-response manner, likely through higher bone area (Strotmeyer et al JBMR 2006). This relationship was not previously examined for sensory PN conduction. PN conduction at the sural sensory nerve (SNAP=amplitude in uV) and peroneal motor nerve (CMAP=amplitude in mV; FWL=mean F-wave latency in ms) were measured with a neurodiagnostic instrument (NC-stat®, NeuroMetrix, Inc.) in 572 community-dwelling men in the Osteoporotic Fractures in Men (MrOS) Study in Pittsburgh, PA. BMD at the total hip and femoral neck (FN), total lean mass (LM) and total fat mass (FM) were measured by DXA (QDR 4500W, Hologic Inc). Participants missing BMD, PN function or diabetes status (DM: self-report, hypoglycemic meds or fasting glucose ≥126 mg/dl) were excluded. In ANCOVA analyses with outcomes of BMD, BMC and area, PN conduction was analyzed as tertiles. Of participants (age 77.5±5.2 years; 99% white), 22% had DM. FN BMD was 4% lower in men with the worst sensory PN conduction compared to the best tertile after adjustment for age, race, diabetes, LM and FM (Table). FN area remained 1% and 3% higher with the worst motor and sensory PN conduction, respectively. Further adjustment for current smoking, drinking frequency, physical activity, calcium or vitamin D supplementation and medication use (corticosteroid, statin, thiazide, thiazolidinedione in DM, and osteoporosis medication) did not change associations. No significant differences were found for PN conduction with FN BMC or for FWL with any bone measure. Our results suggest that the lower BMD in worse sensory PN conduction may be due to an equivalent BMC in a larger hip bone area. Whether differences with worse sensory and motor PN function impact osteoporosis or fracture risk should be investigated in future studies.

FN BMD, BMC and area means by sensory and motor PN function tertile adjusted for age, race, DM, LM,

Motor PN function	**Worst tertile CMAP (≤1.49 mV)**	**Middle tertileCMAP (1.50-3.06 mV)**	**Best tertileCMAP (≥ 3.07 mV)**
FN BMD, g/cm^2	0.783	0.797	0.799
FN BMC, g	4.52	4.55	4.56
FN area, cm^2	5.78†	5.71	5.72
Sensory PN function	**Worst tertile SNAP (≤2.55 uV)**	**Middle tertileSNAP (2.56-4.99 uV)**	**Best tertileSNAP (≥ 5.0 uV)**
FN BMD, g/cm^2	0.769*†	0.797	0.802
FN BMC, g	4.86	4.57	4.53
FN area, cm^2	5.83*†	5.74	5.66

*p<0.05 for worst tertile vs. best tertile; †p<0.05 for worst tertile vs. best tertile at total hip, adjusted for age, race, DM, LM, and FM

Disclosures: ***E.S. Strotmeyer****, None.*

SA329

Osteoporosis in Men: Still Under-Screened and Under-Treated. C. Anastasopoulou, S. Chandrasekaran*, M. David*, A. Chernoff*. Endocrinology, Albert Einstein Medical Center, Philadelphia, PA, USA.

According to the latest National Osteoporosis Foundation recommendations men with hip fractures and all men age 70 and over should be screened for osteoporosis even in the absence of other risk factors. We conducted a retrospective chart review of men who had sustained hip fractures or were over 70. These were patients who were admitted to an urban tertiary care medical center or were followed in the outpatient geriatric and general medicine clinics. The purpose of this study was to determine rates of screening for osteoporosis in men and to assess the impact of known risk factors for osteoporosis in this population. Data collection included demographic information (age, race, height, weight), biochemical values (calcium, creatinine, vitamin D levels) and medical information (results of DEXA bone scan, history of fractures, history of other illnesses including diabetes, thyroid and parathyroid problems, collagen diseases, seizure disorder, COPD, transplant history and the use of specific medications, including steroids, thyroid hormone, statins and antiseizure drugs).

A total of 349 charts were reviewed. There were 178 men with fracture; of these, only 1 had DEXA testing performed (0.6%). Among the men over 70, 6 had had a DEXA Scan (3.5%). Table 1 summarizes data by race.

	White	Black	Hispanic	Asian	Unknown	Total
Patient Number	175	147	12	8	7	349
Total Fracture	103	63	8	4	0	178
Weight recorded	31	30	5	2	0	68
Height recorded	25	34	4	1	0	64
Presence of Diabetes	33	36	2	0	0	71
Mean Age (SD)	74(16)	74(11)	67(20)	60(22)	78(5)	
Mean kgr Weight(SD)	80(24)	82(19)	79(22)	59(8)		
Mean cm Height(SD)	157(50)	144(50)	142(55)	185		

Most of the parameters under consideration were under-recorded whether for special bloods tests like vitamin D, testosterone or intact PTH levels, or the simplest measurements such as weight, height or mobility status. Among patients that suffered bone fractures only 11 (6%) were prescribed calcium and vitamin D supplements, while none were prescribed bisphosphonates. Of the different risk factors for osteoporosis, race (p value 0.024), diabetes (p value 0.046), History of seizure disorder (p value 0.027) and the use of diuretic (p value 0.003) and antiseizure drugs (p value 0.003) were found to be statistically significant on causing bone fractures.

These observations clearly point to a lack of awareness among physicians of the guidelines for osteoporosis screening and treatment in men. More efforts will be needed to increase doctors' awareness of osteoporosis and its treatment in men so that men will be screened for osteoporosis when they have sustained a fracture, if they are over 70 years of age, or younger in the presence of risk factors.

Disclosures: ***C. Anastasopoulou****, Sanofi-Aventis Pharmaceuticals 1; GlaxoSmithKline Pharmaceuticals 1; Procter & Gamble Pharmaceuticals 3.*

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SA330

Ten-year Change in Bone Mineral Density in a Representative Sample of Japanese Women - JPOS Cohort Study. M. Iki[1], J. Tamaki[1], E. Kadowaki*[1], K. Kouda*[1], A. Yura*[1], Y. Ikeda*[2], Y. Sato*[3], A. Morita*[4], S. Kagamimori*[5], Y. Kagawa*[6], H. Yoneshima*[7]. [1]Public Health, Kinki University School of Medicine, Osaka-Sayama, Japan, [2]University Hospital Center for Health and Safety, Kinki University School of Medicine, Osaka-Sayama, Japan, [3]Domestic Sciences, Jinai Women's College, Fukui, Japan, [4]Nutritional Education Program, National Institute of Health and Nutrition, Tokyo, Japan, [5]Welfare Promotion and Epidemiology, University of Toyama, Toyama, Japan, [6]Kagawa Nutrition University, Tokyo, Japan, [7]Shuuwa General Hospital, Kasukabe, Japan.

To describe the change in bone mineral density (BMD) in various ages of a representative sample of Japanese women and to examine whether a cohort effect (difference in BMD of subjects with the same age between surveys conducted in different time) exists in this population.

For 1,651 women aged 15 to 79 years at baseline selected randomly from 3 municipalities in Japan, we measured bone mineral density by DXA at the spine (LS), total hip (TH) and distal 1/3 site of the radius (DR), and body size, and obtained lifestyle factors from in-person interviews. Changes in BMD and other variables were determined 3, 6 and 10 years after the baseline.

Among the cohort from which 133 women dropped out during the 10-year follow-up because of deaths and move of residence, 1040 completed the study. 33% of the subjects were still pre-menopausal at follow-up, 42% were already post-menopausal at baseline, and 18% had entered menopause during the follow-up.

The changes in BMD varied according to ages of subjects. BMD did not show any significant change until 45 years at every skeletal site. After that, an abrupt increase in bone loss occurred at each site. Bone loss at LS decreased with increasing age, that at TH was the greatest in the group aged 75 and older, and that at DR showed a constant rate across different ages. These patterns of bone loss were similarly observed during the first 3 years of follow-up and during the last 4 years.

BMD of a particular age group at 10 years after the baseline was compared with BMD of the same age group at baseline. BMD at follow-up was significantly higher than BMD at baseline in several middle-aged and elderly groups, while no significant difference in BMD was observed in young adults. Height and weight did not differ between the subjects who completed the 10-year follow-up and those who did not. Calcium intake of the former subjects was greater than that of the latter in some age-groups. The observed cohort effect in BMD may have been biased by a healthy people effect. If this bias existed, bone health of young adults may have got worse in these 10 years.

Bone health in Japanese middle-aged and elderly women may have improved in the last decade but that in young adults may not.

Disclosures: ***M. Iki****, None.*

This study received funding from: Japan Society for the Promotion of Science.

SA331

Osteoporosis and the Acid-ash Hypothesis: Evidence Based on Bradford Hill's Criteria for Causality. T. R. Fenton*, A. W. Lyon*, D. A. Hanley, S. C. Tough*, S. Ross*, M. Eliasziw*. University of Calgary, Calgary, AB, Canada.

Scientists from several countries claim that the foods that make up the modern diet cause osteoporosis, due to net acid excretion under the acid-ash hypothesis.
The purpose of this study was to critically evaluate the evidence in the literature regarding the acid-ash hypothesis as a potential cause of osteoporosis.
A systematic review was undertaken based on Hill's criteria of causation: Temporality, Strength, Biological Gradient, Experiment, Consistency, and Biologically Plausibility. Only studies with temporal sequence were included in this review.
Although the quantity of calcium excretion in response to acid loads is sufficient that over a lifetime the loss of calcium could explain the development of osteoporosis (Strength & Biological Gradient), the outcome of urinary calcium may be confounded by changes in absorption. Most studies of the hypothesis have used urinary calcium as the outcome, and Experiments of the hypothesis have failed to include the direct measures of osteoporosis. Prospective observational studies of the association between diet acid loads and changes in bone mineral density have had inConsistent results. Internal Consistency for the hypothesis is weak since there is evidence contrary to the hypothesis regarding the purported roles for phosphate (detrimental) and sodium (protective) in terms of calcium excretion, and no clear evidence for the hypothesis purported deleterious effects of grains and organic acid containing fruits on bone health. Biological Plausibility of the acid-ash hypothesis is weak based on the in-vitro studies that indicated increased bone demineralization occurs at low pHs since these studies were conducted at pHs below the physiological range. The use of Hill's criteria to evaluate the relationship between the acid-ash hypothesis and osteoporosis indicates that the evidence to support this hypothesis is weak.

***Disclosures: T.R. Fenton**, None.*
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SA332

Glomerular Filtration Rate and Physical Function in Community-Dwelling Japanese Frail Elderlies. J. Okuno*, S. Tomura*, H. Yanagi*, N. Yabushita*, T. Okura*, K. Tanaka*. Graduate school of Comprehensive Human Sciences, University of Tsukuba, Tsukuba city, Japan.

Chronic kidney disease (CKD) is becoming increasingly recognized as an important comorbid condition in elderly individuals. The risk of falling increases with aging. Falls are associated with deterioration of quality life. Vitamin D deficiency associated with falls and decreased balance. Impaired renal function with aging is detrimental for the conversion of calcidiol to calcitriol (D-hormone) and D-hormone analogue have been shown to decrease the risk of falls. The aim of this study was to assess whether estimated glomerular filtration rate (eGFR, ml/min/1.73m^2) is associated with physical function in 51 community-dwelling frail elderly enrolled a three-month exercise program for nursing care prevention (76.9±5.9 yr). A longitudinal study conducted in a town near Tsukuba city (latitude 36°north) from June to September in 2006 and 2007 and November to February in 2006. The Ethics Committee of University of Tsukuba approved the study. An interview was conducted based on a questionnaire including experiences of fall or stumbling during the past one year. The serum levels of 25(OH)D, intact parathyroid hormone (iPTH) 1,25-dihydroxyvitamin D (1,25$(OH)_2$D), calcium, and creatinine were measured. The following physical tests were measured: timed up and go (TUG), a 5-meter walk, functional reach (FR), one-legged stance with open eyes, tandem stance, tandem walk, hand grip strength, and alternate step-test. Estimated GFR was calculated using the Modification of Diet in Renal Disease (MDRD) formula (<60 versus >=60 ml/min/1.73m^2). The rate of participants with GFR<60 was 23.5%. At baseline, age, iPTH, 25(OH)D, and 1,25$(OH)_2$D were not different between those with GFR<60 and those with GFR>=60. All physical functions were not different between two groups at baseline and at three months later. Physical functions except FR and hand grip strength improved significantly in those with GFR>=60. On the hand, many of physical functions did not improved in those with GFR<60. GFR were associated with iPTH (P<0.1), iPTH significantly correlated with 25(OH)D (P<0.05), and 25(OH)D significantly correlated with 1,25$(OH)_2$D. Although the rate of 25(OH)D<50 nmol/L was not different between two groups, the rate of 1,25$(OH)_2$D<42 pg/ml (lowest quartile) in the subjects with GFR<60 was higher than those with GFR>=60, but, not significant (50.0% vs 19.5%). Tandem stance, 5 chair sit to stand, and alternate step-test were significantly affected by GFR by multiple liner regression analysis (β:0.30, -0.32, -0.30, respectively). Our data suggest that eGFR is highly associated with walking ability and balance.

***Disclosures: J. Okuno**, None.*

SA333

See Friday Plenary number F333.

SA334

Seasonal Genetic Influence on Serum 25-hydroxyvitamin D Levels. G. Snellman*[1], H. Melhus[2], R. Gedeborg*[3], S. Olofsson*[4], A. Wolk*[5], N. Pedersen*[6], K. Mïchaëlsson[1]. [1]Department of Surgical Sciences Section of Orthopedics, Uppsala, Sweden, [2]Department of Medical Sciences Section of Clinical Pharmacology, Uppsala, Sweden, [3]Department of Surgical Sciences Section of Anaesthesiology and Intensive Care, Uppsala, Sweden, [4]Clinical Research Center, Uppsala, Sweden, [5]Division of Nutritional Epidemiology, Institute of Environmental Medicine, Stockholm, Sweden, [6]Department of Epidemiology and Biostatistics, Stockholm, Sweden.

Environmental factors, mainly nutrition and UV-B radiation, have been considered major determinants of vitamin D status. Nevertheless, they have only explained a modest proportion of the variation in serum 25-OH vitamin D. We aim to present the seasonal genetic impact on serum 25-OH vitamin D levels, an issue not previously resolved, and to illustrate the variability and reliability between three different 25-OH vitamin D assays.
204 same-sex twins were recruited from The Swedish Twin Registry. Serum 25-OH vitamin D was analyzed in three different laboratories, using three different techniques: high performance liquid chromatography and mass spectrometry (HPLC-MS), a radioimmunoassay (RIA), and a chemiluminescent immunoassay (CLIA). The heritability was calculated using genetic modelling techniques to estimate the relative contributions of genetic, shared and non-shared environmental factors to the variation in serum vitamin D.
Our results show high variability in 25-OH vitamin D assay results between the methods. There is a 24.9 nmol/l difference in mean 25-OH vitamin D level between the highest (HPLC-MS) and the lowest (CLIA) values. With a 50 nmol/L cut-off, only 8% of our twins are classified as vitamin D deficient with the HPLC method, 22% with the RIA method but 43% with the CLIA method. Lowest coefficient of variation was observed for the HPLC-MS method. The seasonal variation in heritability of vitamin D status is substantial. When using the HPLC-MS assay results, as much as 76% (95% CI 37-87%) of the variability in 25-OH vitamin D during the warm season (May through October) is explained by genetic factors. On the contrary, the seasonal variation in serum 25-hydroxyvitamin D is largely attributable to shared environmental influences, i.e. solar altitude. Individual environmental influences are found to only explain approximately one fourth of the variation in serum 25-OH vitamin D independent of season.
Our results indicate a strong genetic impact on serum vitamin D status during the warm season. The most likely main contributor to this genetic influence is the skin synthesis of vitamin D. Further studies are warranted to identify genes controlling vitamin D status. The high variability in results between the different serum 25-OH vitamin D assays may lead to substantial misclassification of vitamin D status.

***Disclosures: G. Snellman**, None.*

SA335

See Friday Plenary number F335.

SA336

Soy-based Formula Promotes Bone Growth in Neonatal Piglets by Inducing Osteo-progenitors to Differentiate into Osteoblasts via Enhanced BMP2 Signaling. J. R. Chen*[1], O. P. Lazarenko*[2], M. L. Blackburn*[2], T. M. Badger*[2], M. J. Ronis*[1]. [1]Pharmacology & Toxicology, University of Arkansas for Medical Sciences / Arkansas Children's Nutrition Center, Little Rock, AR, USA, [2]Physiology, University of Arkansas for Medical Sciences / Arkansas Children's Nutrition Center, Little Rock, AR, USA.

Despite consumption of soy infant formula by 20% of infants in the United States and of soy products by children in most Asian countries, the majority of studies of soy effects on bone in both human and experimental animal models have focused on adults, particularly postmenopausal females. There have been no studies conducted on soy effects on immediate postnatal bone growth and maintenance. Moreover, the molecular mechanisms underlying soy effects have not been well elucidated. In the current study we used neonatal piglets as a model of human infants. Both male and female piglets were breast-fed (BF) or fed dairy-based formula (MF) or soy-based formula (SF) (n = 6/group) from birth until postnatal day 35. After sacrifice, blood, bone, bone marrow and other target tissues were collected for analysis. Bone quality and growth rate were assessed by peripheral quantitative computerized tomography and dynamic histomorphometry of the left tibia. Results showed that bone mineral density, content, and cortical thickness were all greater (P<0.05) in the SF group compared to the BF group. The MF group had minor effects compared to BF. Osteoblast numbers and bone formation rate were increased (P<0.05) in the SF group when compared to BF group; whereas, osteoclast numbers were decreased. Osteoblastogenesis was increased (P<0.05) in the SF group in *ex vivo* bone marrow cell cultures. Alkaline phosphatase and osteocalcin, bone formation markers in serum, were increased (P<0.05); whereas, the bone resorption marker CrossLaps was decreased in the SF group compared to BF group. Real-Time PCR results demonstrated that BMP2 was up-regulated, but RANKL was down-regulated in the SF group compared to BF group (P<0.05). Western Blots showed up-regulation of ERK, p38, Smad1/5/8 and RUNX2 from bone samples in both SF and MF groups compared to BF group (P<0.05). Treatment of C2C12 and ST2 cells with 2.5% serum from SF piglets showed increased (P<0.05) osteoblast differentiation compared to serum from BF piglets. These data indicate that SF has significant effects on promotion of bone growth, and these effects are mediated through enhancing of BMP2 signaling leading to increased osteoblast differentiation. *Supported in part by USDA ARS 6251-51000-005-02S-2.*

***Disclosures: J.R. Chen**, None.*

SA337

See Friday Plenary number F337.

SA338

Weight Reduction Is Not Deleterious for Bone Mass or Strength in Obese Women. K. Uusi-Rasi, H. Sievänen, P. Kannus*, M. Pasanen*, K. Kukkonen-Harjula*, M. Fogelholm*. UKK Institute for Health promotion Research, Tampere, Finland.

Obesity is associated with greater bone mass. Therefore, one deleterious consequence of weight loss might be bone loss and increased bone fragility. This was a prospective 12-mo study with 3-mo weight loss following a 9-mo weight maintenance period. All 55 participants were premenopausal women with the mean age (SD) of 40 (5) years and BMI of 33.6 (4.7). All were encouraged to use VLED-products (Cambridge Diet) in reducing body mass. In addition, use of vegetables and energy-free drinks were recommended. The mean daily use of products was 2.8 (0.7) bags providing 41 (10) g of protein and 862 (216) mg of calcium per day from the products.

Body composition and bone mineral mass and strength of the right proximal femur and lumbar spine were assessed by DXA. Cortical (shaft CoD) and trabecular bone density (distal TrD) of the radius and tibia were measured with pQCT. All assessments were done at baseline and 3 and 12 months afterwards. The participants were divided into 5 equal groups (n=11; Q1-Q5) by the absolute loss of body mass during the 3-mo dieting period (Table). Analysis of variance (ANOVA) was used to find between-group difference and linearity in changes of bone characteristics during the 12-mo period.

Absolute changes in bone values were tiny and mostly not associated with weight loss (Table). The between-group differences in changes were statistically significant in CoD of the radial shaft only (p=0.020), and borderline difference was found in femoral neck BMD (p=0.063) and BMC (p=0.088). Only the mean changes in BMD of the femoral neck and trochanter showed a statistically significant linearity between weight loss groups (p=0.035 and 0.036, respectively). At the tibia or spine, there were no significant changes in bone traits in any groups. Even the greatest weight loss (group Q5) did not seem to be harmful for bone mass during a 12-mo time period. Although the Q5 showed a decline in femoral neck BMC, the change was insignificant. Simultaneously total body BMC and radial shaft CoD increased in this group.

The greater weight reduction did not seem to result in greater bone loss compared with minor weight reduction. Sufficient intake of calcium and protein were ensured during dieting, which may partly explain good maintenance of bone mass.

Mean (SD) %-changes of bone traits in weight loss groups during 12 months

Weight loss groups, mean decline (kg)	Total body BMC	Lumbar spine BMC	Femoral neck BMC	Radial shaft CoD
Q1=4.1 (2.7)	-0.7 (2.9)	0.4 (3.1)	1.2 (3.0)	-0.1 (0.7)
Q2=7.3 (0.4)	-0.6 (4.0)	1.2 (3.7)	1.9 (3.3)	0.4 (0.8)
Q3=9.9 (1.0)	-1.4 (4.3)	-0.4 (3.4)	-0.4 (3.4)	-0.4 (0.7)
Q4=12.9 (0.9)	-0.9 (3.9)	-0.8 (5.9)	1.3 (2.7)	-0.03 (0.7)
Q5=16.3 (1.8)	1.1 (6.0)	-0.4 (3.7)	-1.0 (2.4)	0.8 (1.1)
P for between-group diff.	0.72	0.65	0.088	0.02
P for linearity	0.33	0.23	0.06	0.12

***Disclosures:** **K. Uusi-Rasi**, None.*

SA339

See Friday Plenary number F339.

SA340

Bone Density (BMD), Fracture and Survival in Women in Nursing/ Residential Care. M. N. Dugard*, T. J. Jones*, M. W. J. Davie. Charles Salt Research Centre, Robert Jones & Agnes Hunt Orthopaedic Hospital NHS Trust, Oswestry, United Kingdom.

Fractures, a common event in nursing homes (NH/RH) are often associated with low BMD. Because fractures may occur early after entry and drug treatment may take up to 18 month for effect, different strategies for fracture prevention may be required according to survival. To investigate BMD fractures and survival we restudied 156 women in NH/RH aged 56.4-98.8yr screened for fracture risk 3-8yr previously. A questionnaire was completed on all women at the first visit and distal forearm BMD (DTX 100) measured in 137. BMD was expressed as g/cm^2 or as a z score. Women were traced through health records, fracture details being obtained from the each woman's doctor. There were 7 <69yr, 26 70-79yr and 104 women aged >80yr at first interview.

BMD measured 1.3yr (range 0.05-9.3yr) after entry into NH/RH was low (0.317±0.084g/cm^2, p<0.001). Osteoporosis (BMD<0.34 g/cm^2) existed in 72.1% of women aged >80yr, compared with 33.4% less than 80yr (p<0.001). Body weight was lower in those >80yr compared with younger women (56.1±12.5 vs 62.3±16.7kg, p<0.05). Cross sectional analysis showed that BMD or body weight <6 months after entering a NH/RH (BMD 0.335±0.085g/cm^2, weight 57.5±13.4kg) was similar to those >1yr after entry (BMD 0.317±0.082, 58.4±15.1kg). At follow-up 83.9% had died, 14.3% >69yr, 73.1% 70-79yr and 93.1% >80yr. Median survival time after entering a NH/RH was 4.6yr (range 0.4-15.3yr) in all subjects and 4.2yr (0.4-15.3yr) in the >80yr age group. In a Cox proportional hazards model, shortened survival in a NH/RH was associated with lower BMD z score (HR 1.42; 95% CI 1.04-1.93) in women >80yr scanned within 1yr of entry but not by age at entry or weight. Few fractures occurred in women <70yr. Fracture analysis has been performed on 56 women >80yr (268.2 person yr). There were 22 fractures in 17 women in 4.12yr (Median: range 0.9-15.3yr), the first being 2.5yr (0.1-6.7yr) post entry. Seven women sustained fracture within 1yr of entry. Previous fracture was not a risk factor for future fracture. Post entry fracture rate was 8.2/100 person yr (1.5/100 for hip fracture). BMD was low in 3/4 women sustaining hip fracture (z score -0.474±1.089) and 9/13 women with non-hip fracture (-0.364±0.909) that occurred after entry.Most women in NH/RH have osteoporosis. In those over 80yr low BMD is associated with shorter survival. Fractures are associated with low BMD and may occur soon after entry. BMD measurement should form part of the initial entry assessment. Median survival times indicate that most women will survive long enough to benefit from bisphosphonate therapy.

***Disclosures:** **M.W.J. Davie**, MSD 2, 3; Procter & Gamble 4; Servier 3.*
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SA341

See Friday Plenary number F341.

SA342

Effects of Obesity on Cortical Bone. S. Ionova*[1], S. H. Do*[2], H. D. Barth*[1], J. W. Ager*[1], A. Porter*[3], C. Vaisse*[2], T. Alliston[2], R. O. Ritchie*[1]. [1]Material Science Division, Lawrence Berkeley National Laboratory, Berkeley, CA, USA, [2]University of California San Francisco, San Francisco, CA, USA, [3]Imperial College London, London, United Kingdom.

Obesity is associated with a host of biological and physiological changes, among which is a reduced risk of bone fracture in adults. While some studies have found obesity is associated with increased bone size and mass measures, it is still unclear whether the reduced risk stems from a change in bone quantity alone, or whether bone quality is affected as well. The objective of this study is to evaluate the changes in mechanical properties of cortical bone in response to diet-induced obesity in mice. 4 week old C57BL/6 male mice were fed a high fat diet (HFD) (N=15) or standard laboratory chow (N=15) for 16 weeks. All protocols were approved by the Institutional Animal Care and Use Committee and done according to federal guidelines for the care and use of animals in research. Blood was isolated immediately following sacrifice to evaluate serum leptin levels. Bone mass and body composition were evaluated with DEXA. The left femurs were tested in three-point bending to measure strength and bending stiffness. Right femurs were tested in notched three-point bending to measure fracture toughness (loading rate of 0.001mm/s). Bone geometry was evaluated in tomography at the Advanced Light Source and in an environmental scanning electron microscope. As expected, in the high-fat diet fed mice, body weight, fat mass, and leptin levels were significantly increased. In HFD mice, endostial and periostial diameters, as well as cortical wall thickness increased (all p<0.015), while BMD was unchanged (p=.937). Strength, bending stiffness, and fracture toughness all were reduced in HFD (p<0.007, p=.010, p=.013, respectively). Transmission electron microscope studies point to a qualitative reduction in collagenous organization in HFD versus control group. In summary, diet-induced obesity results in increased bone size (quantity), while reducing bone strength, bending stiffness, and fracture toughness as well as other indicators of bone quality. This study indicates that bone quantity and bone quality play important, albeit counteracting, roles in determining fracture risk.

***Disclosures:** **S. Ionova**, None.*
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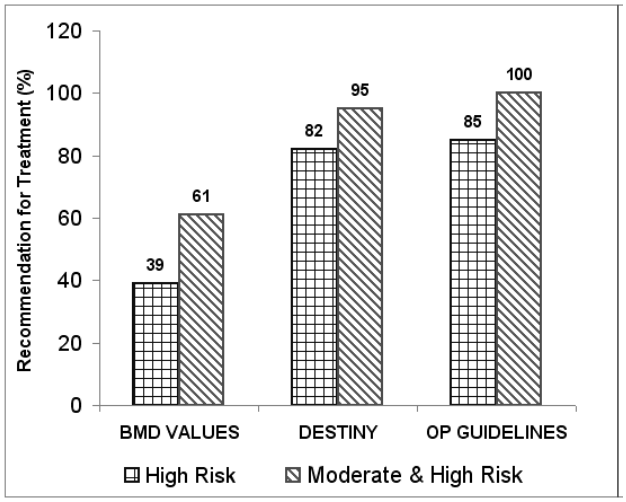
SA343

Patients with Fragility Fracture Assessed by BMD, Bone DESTINY and Osteoporosis Canada Guidelines. M. Larche*, K. Beattie*, J. D. Adachi, A. Papaioannou, W. Wong Pack*, W. G. Bensen*. Dept. of Medicine, McMaster University, Hamilton, ON, Canada.

Purpose: Fracture risk and treatment recommendations were compared in female patients with a previous fragility fracture using bone mineral density (BMD), Bone DESTINY and Osteoporosis Canada (OC) guidelines.
Methods: All female patients >60 years of age with a previous fragility fracture who underwent a DEXA scan between June and November 2007 were included in the analyses. Fracture risk and treatment recommendations were made based on BMD alone, Bone DESTINY and Osteoporosis Canada (OC) guidelines and results were compared. DESTINY fracture risk was an integration of BMD, age, steroid use, propensity to fall, history of falls and BMI<20 kg/m^2 while OC guidelines included BMD, age and steroid use. The prevalence of women in the high fracture risk group was compared between these three methods.
Results: 840 female patients with a previous fragility fracture were included in the analyses. BMD values resulted in treatment recommendations for 39% of patients with a T-score <-2.4 and 61% of patients at a T-score of -2 as shown in Figure 1. Bone DESTINY suggested treatment in 82% of patients with an additional 13% of patients in the moderate risk zone also seen in Figure 1. According to the OC guidelines, 85% of patients were in the high-risk zone with all patients being in the moderate and high risk zones.
Figure 1. Recommendations for treatment according to BMD, Bone DESTINY and OC guidelines.



Conclusions: Bone DESTINY suggests treatment in a much higher number of fragility fracture patients than BMD alone (82% versus 39% at -2.5), a value which is similar to OC guidelines. Based on the agreement in results between DESTINY and OC guidelines, the simplicity and ease of interpretation make DESTINY an attractive option for reporting fracture risk.

***Disclosures: K. Beattie**, None.*

SA344

Long Term Safety of Oral Risedronate Treatment in Men with Osteoporosis as Assessed by Histomorphometry. R. Recker[1], R. Eusebio*[2], R. Phipps[2], S. Xu[2], D. Vanderschueren*[3], S. Boonen[3]. [1]Osteoporosis Research Center, Creighton University, Omaha, NE, USA, [2]Procter & Gamble, Mason, OH, USA, [3]University Hospital Leuven, Leuven, Belgium.

The effects of 2-4 years of 35 mg once-a-week risedronate treatment on bone quality and bone remodeling were assessed in a 2-year double-blind, placebo-controlled study in men with osteoporosis, followed by a 2-year open-label extension in a subset of subjects who all received risedronate. In the double-blind study, transiliac bone biopsies were obtained after 2-years treatment with placebo (N=7), and risedronate (N=15). During the open label study, 4 of the 7 placebo patients had a second biopsy taken after 2 years of risedronate treatment (total=19), and 9 of the 15 risedronate patients had a second biopsy taken after 4 years of risedronate treatment. There were no significant differences among groups in demographics or in any baseline characteristics including bone mineral density and bone turnover markers. Biopsies were examined for qualitative histological and quantitative histomorphometric differences. All biopsies had lamellar bone structure with no signs of osteomalacia, woven bone or bone marrow fibrosis. Tetracycline label was observed in all samples. Bone remodeling rate as expressed as mineralizing surface/bone surface was significantly lower after 2 and 4 years treatment with risedronate compared to 2 years placebo. Median eroded surface/bone surface (bone resorption parameter) was 2.8% in placebo and 1.7% after 2 years risedronate treatment (p=0.027). Median mineralizing surface/bone surface (bone formation parameter) was 3.6% after 2 years placebo, 1.2% after 2 years risedronate (p=0.0207), and 1.1% after 4 years risedronate treatment. These results indicate continued bone formation and no excessive inhibition of bone turnover with 2 and 4 years treatment with risedronate. There were no significant differences in structural parameters with treatment. These results are concordant with previous histological studies with risedronate in postmenopausal women with osteoporosis showing that risedronate preserves normal bone structure, quality, and remodeling. This favorable long-term bone safety profile is consistent with that demonstrated in clinical trials in women with postmenopausal osteoporosis.

***Disclosures: R. Recker**, P&G 2.*
This study received funding from: The Alliance for Better Bone Health (Procter & Gamble Pharmaceuticals and sanofi-aventis).

SA345

See Friday Plenary number F345.

SA346

Serum Vitamin D Levels Do Not Modify the Response to an Exercise Program Following Hip Fracture: Data from the Baltimore Hip Studies. M. C. Hochberg[1], E. Streeten[1], J. Yu-Yahiro[2], W. Hawkes*[3], R. Hebel*[3], R. Miller*[3], D. Orwig*[3], J. Magaziner[3]. [1]Medicine, University of Maryland, Baltimore, MD, USA, [2]Orthopaedic Surgery, Union Memorial Hospital, Baltimore, MD, USA, [3]Epidemiology and Preventive Medicine, University of Maryland, Baltimore, MD, USA.

Background: While older women with hip fracture often have low serum 25-hydroxyvitamin D (25[OH]D) levels, it is unknown whether recovery after hip fracture is associated with serum 25(OH)D levels.
Objective: To assess whether changes in BMD, muscle strength and lower extremity performance (LEGS Score) that occur in the year following hip fracture in response to a home exercise program were modified by baseline serum 25(OH)D levels.
Methods: We performed a post-hoc analysis of data from BHS-4, a randomized controlled trial of a home exercise program in 180 community-dwelling older women (mean [SD] age 82.3 [7.0] years) who had been hospitalized for surgical treatment of a hip fracture. Hospitalized women were recruited and baseline data were acquired within 15 days of fracture. BMD at the contralateral hip and grip strength were measured, LEGS Score was assessed and serum was collected at baseline, 2, 6 and 12 months post fracture. Serum samples stored at -70C were analyzed for 25(OH)D levels in duplicate using DiaSorin RIA. Women were divided into two strata based on serum 25(OH)D levels either below 20 ng/ml or 20 ng/ml or above. Generalized estimating equations, with time and intervention as fixed effects, were used to examine whether 25(OH)D levels modified the effect of exercise on the outcomes.
Results: Baseline serum samples from 96 women were assayed for 25(OH)D levels. The mean (SD) 25(OH)D level was 22.8 (13.7) ng/ml and the median was 21.6 ng/ml with an interquartile range of 12.6 to 30.4 ng/ml; 45 (47%) women had a 25(OH)D level below 20 ng/ml. There was no evidence of seasonal variation in baseline 25(OH)D levels. There was no evidence of a significant effect of the home exercise intervention on any of the outcomes in women with 25(OH)D levels either above or below 20 ng/ml; results in Table.
Conclusion: These data fail to demonstrate that serum 25(OH)D levels modify the effect of a home exercise program on changes in BMD, grip strength and lower extremity performance in the year following hip fracture. Results are limited by small numbers and the lack of ability to examine the potential efficacy of supplementation with vitamin D3 during the year following the fracture.

BMD, Grip Strength and LEGS Score at 2, 6 and 12 Months after Hip Fracture by Serum 25(OH)D Levels

Outcome	Group	2 Months	6 Months	12 Months
		Low 25(OH)D Normal 25(OH)D	Low 25(OH)D Normal 25(OH)D	Low 25(OH)D Normal 25(OH)D
Total Hip BMD, g/cm2	Exercise	0.641 (0.025) 0.631 (0.025)	0.636 (0.025) 0.619 (0.024)	0.633 (0.024) 0.616 (0.027)
	Control	0.651 (0.025) 0.672 (0.026)	0.629 (0.027) 0.680 (0.027)	0.635 (0.023) 0.671 (0.031)
Grip Strength, kg	Exercise	16.8 (1.0) 17.4 (0.8)	16.6 (1.0) 17.5 (0.8)	16.7 (1.0) 17.6 (1.0)
	Control	17.8 (1.0) 17.9 (1.3)	16.5 (1.0) 16.6 (1.4)	16.6 (0.9) 18.1 (1.3)
LEGS Score	Exercise	22.4 (2.3) 25.1 (1.1)	25.3 (2.0) 28.3 (0.8)	31.4 (1.1) 32.5 (0.7)
	Control	19.9 (1.9) 25.7 (1.6)	23.5 (2.0) 29.5 (1.2)	26.5 (2.0) 33.4 (0.8)

***Disclosures: M.C. Hochberg**, None.*
This study received funding from: National Institutes of Health

SA347

See Friday Plenary number F347.

SA348

Excess of Post-fracture Mortality Among Men and Women: A Relative Survival Analysis. S. A. Frost*, N. D. Nguyen, J. R. Center, J. A. Eisman, T. V. Nguyen. Bone and Mineral Research Group, Garvan Institute of Medical Research, Sydney, Australia.

Although it is now well-established that individuals with fracture are at substantial increased risk of mortality, it is not clear how many deaths can be attributed to fracture. It can however be hypothesized that the excess deaths related to osteoporotic fracture are due to two "causes": one due to fracture and the other due to non-fracture. Therefore, if the expected background mortality rate reflects the effect of "other causes" then it is possible to estimate the excess of mortality due to osteoporotic fracture. The aims of this study were therefore (a) to estimate the "relative survival" among men and women following an osteoporotic fracture, and (b) to estimate the excess of mortality associated with specific fracture site.

The Dubbo Osteoporosis Epidemiological Study was designed as a prospective epidemiologic investigation in which men and women aged 60+ as of 1989 had been followed for 19 years. During this period incidence of atraumatic fractures ascertained by X-ray reports, and mortality was ascertained by personal report and confirmed by the New South Wales Birth, Death and Marriage Registry.

Relative survival can be illustrated by the following comparison: a 60 year-old individual died after a fracture in 2006 is considered to incur a greater loss of life than an individual with the same age died after a fracture in 1980, because the population life expectancy in 2006 is greater than that in 1980. Therefore, relative survival analysis allows a better estimate of the true effect of fracture on mortality. In this study relative survival ratio (RSR) was estimated by taking into account the age-and-sex specific expected survival in the general Australian population.

During the follow-up 585 women and 230 men sustained an incident low-trauma fracture. Death occurred in 138 (24%) women and 108 (47%) men. In men, the RSR of post-fracture mortality at 1, 5 and 10-year was 0.93 (95% CI 0.88 - 0.98), 0.83 (95% CI 0.71 - 0.96), and 0.80 (95% CI 0.56 - 1.05), respectively; in women, the corresponding estimates were: 0.99 (95% CI 0.97 - 1.01), 0.98 (95% CI 0.94 - 1.04), and 0.93 (95% CI 0.81 - 1.04). In both men and women, hip and vertebral fractures were associated with lower RSR. In comparison to forearm fracture, men and women with hip fracture had an 86% and 81%, respectively, increase in relative excess mortality. Men and women with vertebral fractures had a 23% and 96%, respectively, increase in relative excess mortality compared to forearm fractures.

In summary, relative survival analysis indicated that men had a worse outcome than women because the post-fracture risk of death in men was greater in men than in women. In both sexes the excess of mortality associated with osteoporotic fractures was mainly due hip and vertebral fractures.

***Disclosures:** **S.A. Frost**, None.*

SA349

See Friday Plenary number F349.

SA350

Performance Characteristics of a Workflow Tool for the Assessment of Vertebral Shape. T. Ozanian*, T. Lacey*, G. Zubko*, A. Brett*, P. Steiger. Optasia Medical, Inc., Burlington, MA, USA.

The purpose of this study is to assess the performance of a new workflow tool based on statistical shape modelling for the characterization of vertebral shape.

We previously described a novel method for the computer-assisted determination of vertebral shape using 95 points representing the circumferential vertebral borders on lateral spine x-rays[1]. We implemented this method in a computer program that allowed an operator to annotate vertebral shapes on lateral radiographs from T4 to L4 as follows: (1) Choose image, (2) place a single point at the approximate centre of each vertebra to be analyzed, (3) using an atlas, label anatomy being analyzed and initialize automated search of vertebral contours, (4) review and correct contours. We assessed effort by measuring the time elapsed for steps 1-4. Four non-expert operators performed the analysis twice on 10 subjects after having been trained on 5 different training subjects. We then compared the results of each operator with those of a reference mark-up by measuring the average distance of all placed points (95 per vertebra, 13 vertebra per subject, 10 subjects = 12,350 points) to the curves defined by reference points. We also measured the ability to repeatedly produce accurate results (precision) by the different operators (inter-operator precision) and by the same operators (intra-operator precision).

	Results		
All values mean (SD)	Effort [mins] per patient	Accuracy [mm] per vertebra	Intra-Operator Precision [mm] per vertebra
Operator 1	8.9 (1.9)	0.92 (0.43)	0.97 (1.25)
Operator 2	7.4 (1.6)	0.85 (0.33)	0.63 (0.71)
Operator 3	6.2 (1.3)	0.85 (0.31)	0.63 (0.93)
Operator 4	6.9 (2.8)	0.91 (0.38)	0.61 (0.86)
Overall	7.4 (1.9)	0.88 (0.36)	0.71 (0.94)

Inter-operator precision was 0.91 (0.92) mm.

These results indicate that the accurate and reproducible computer assisted annotation of vertebral body shape can be accomplished within time constraints that are feasible in a point-of-care setting. The operator error was around 1 mm, which corresponds to about 3% of a vertebral height and compares favourably to the current guidelines for clinical trials, which specify that changes of 3-4mm or 15-20% constitute vertebral fractures. Prevalent vertebral fractures are important predictors of future osteoporotic fractures in the spine and hip. This method may prove useful as a workflow tool to aid the physician to quantify vertebral shape. Further work is needed to explore how it may assist physicians in the assessment and classification of vertebral fractures. If successful, this tool could help standardize vertebral fracture assessment and make accurate quantitative assessment of vertebral shape feasible in a point-of-care setting.

[1]Brett et al. In Proc ASBMR, W227, Hawaii, Sept 2007.

***Disclosures:** **P. Steiger**, Optasia Medical, Inc. 5.*
This study received funding from: Optasia Medical, Inc.

SA351

See Friday Plenary number F351.

SA352

Risks and Outcomes for Hip Fractures in a Predominantly African-American Male Veteran Population. R. U. Hashmi*[1], A. Rahman*[2], F. P. Perez*[3], S. C. Kukreja*[1], E. I. Barengolts[3]. [1]Medicine, University of Illinois at Chicago, Chicago, IL, USA, [2]Medicine, Weiss Memorial Hospital, Chicago, IL, USA, [3]Medicine, Jesse Brown VA Medical Center, Chicago, IL, USA.

The risks and outcomes associated with hip fractures are not well characterized in male veteran population. In this study we reviewed charts of patients admitted to VA Chicago hospital for hip fracture and for hip replacement surgery from January 2000 to December 2002. This preliminary report includes analysis of 33 age-matched subjects from each group (Table). There was no difference in smoking and alcohol abuse between the groups. DXA was done in 4 (12%) and bisphosphonates were used in 2 (6%) patients with fractures.

Table. Characteristics of cases (hip fracture) and controls (hip replacement).

Variable	**Cases**	**Controls**	**P Value**
Numberof Subjects	33	33	x
African Americans, %	62	58	0.7
Age, years ± SD	69.8±7.7	69.2±6.0	0.7
Outcomes	**Cases**	**Controls**	**P Value**
Outcome[1]	1.6± 0.6	1.2 ±0.4	<0.0004
Mortality in hospital, %	6.2	2.0	0.5
Mortality 1 year, %	28.1	6.5	0.02
Morbidity in hospital[2],%	30.3	9.1	0.03
Co-morbid conditions,N±SD	6.2 ±3.2	4.2 ±2.4	0.004
Concomitant medication,N±SD	8.0 ±3.7	4.3 ±2.8	<0.0001
Hospitalization, days± SD	7.4±13.4	2.3± 8.6	0.07
Risk Factors	**Cases**	**Controls**	**P Value**
Seizure disorder, %	15.2	0.0	0.02
Stroke, %	21.2	9.1	0.17
Depression, %	21.2	9.1	0.17
COPD, %	15.2	3.0	0.08
Anti-seizure medication, %	18.2	0.0	0.01
Anti-depressants, %	33.3	9.1	0.02
Calcium supplements, %	36.4	6.1	0.003

[1] Outcome: 1=discharge home, 2=discharge to nursing home, 3=death
[2] Morbidity in the hospital included pneumonia, stroke and falls
[3] Hospitalization during 1 year prior to fracture

There was a high prevalence of seizure disorder, stroke and depression and in-hospital and 1 year mortality was 3-4 fold higher in patients with hip fractures than those with hip replacement. Our data suggest that patients with hip fractures have high prevalence of co-morbid, particularly neurological conditions and poor outcomes. If these data are confirmed, interventions to prevent hip fractures in high risk population with neurological disorders may be warranted.

***Disclosures:** **R.U. Hashmi**, None.*

SA353

Impact of an Educational Intervention on Knowledge about Osteoporosis Following a Fragility Fracture. L. Bessette[1], S. K. Davison[1], L. Ste-Marie[2], S. Jean*[3], M. Beaulieu*[4], M. Baranci*[5], J. P. Brown[1]. [1]CHUL Research Centre, Quebec, QC, Canada, [2]Medecine, University of Montreal, Montreal, QC, Canada, [3]Institut National de Santé Publique du Québec, Quebec, QC, Canada, [4]Merck Frosst, Kirkland, QC, Canada, [5]sanofi-aventis, Laval, QC, Canada.

The objective of this analysis was to evaluate the impact of an educational intervention on general osteoporosis (OP) knowledge in women ≥50 years who recently suffered a fragility fracture.
Three educational tools were developed: a 15-minute video, written material on OP targeted to participants, and written documentation on OP targeted to physicians. Information was based on the 2002 Clinical Practice Guidelines for the Diagnosis and Management of Osteoporosis in Canada. Six to eight months after the fracture event, women with a fragility fracture were contacted by phone and offered to be randomized to one of three groups: A) the control group (no intervention); B) the documentation group (written documents for the participants and the physician); or C) the video group (a video and the written documentation for both the participants and the physician). All participants completed a phone questionnaire on OP, which included a component on OP knowledge, before the intervention. Twelve months after the intervention participants were re-contacted and the same questionnaire was administered. The current analysis was completed only for participants who were undiagnosed and untreated for OP at randomisation.
Of the 894 women with a fragility fracture, 850 (95%) accepted randomization. Of these, 546 (64%;mean age: 61.7 years) were without OP diagnosis and treatment. Ninety-six percent of these 546 women were contacted and completed the phone questionnaire 12 months after the randomization. The mean knowledge score improved significantly from baseline in both groups B and C (81.6 to 85.5% and, 79.7 to 84.4%, respectively) compared to group A for whom there was little average change (79.9% to 81.9%). As showed in the table, groups B and C improved their knowledge between baseline and 12 month follow-up more so than the group A.
A simple intervention targeting the patient increased general knowledge about OP and its consequences.

% strongly agree or agree with the statement	Groupe A Baseline/12 mo	Group B Baseline/12 mo	Group C Baseline/12 mo
I understand the possible consequences of the fracture on my health well	84.2/85.6%	83.1/92.5%	83.9/91.6%
I know OP and its consequences well	50.0/58.1%	51.1/74.1%	51.6/77.0%
I understand the relationship between OP and my fracture well	61.8/66.8%	66.6/71.9%	57.5/71.0%
I know the treatments for OP well	19.5/24.8%	19.8/38.7%	21.9/45.1%

Disclosures: ***L. Bessette****, Procter & Gamble, Sanofi-Aventis 1, 3; Merck Frosst 1, 3; Amgen 1, 2, 3; Pfizer 1, 2, 3.*
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SA354

See Friday Plenary number F354.

SA355

Gender Specific Effects of TRPV4 on Osteoblast-Osteoclast Coupling and Risk of Osteoporotic Fractures. B. C. J. van der Eerden*[1], M. Koedam*[1], F. Rivadeneira[1], J. B. J. van Meurs[1], J. G. J. Hoenderop*[2], H. Weinans*[3], M. Suzuki*[4], R. J. M. Bindels*[2], A. G. Uitterlinden[1], J. P. T. M. van Leeuwen[1]. [1]Internal Medicine, Erasmus MC, Rotterdam, Netherlands, [2]Cell Physiology, Nijmegen Centre for Molecular Life Sciences, Nijmegen, Netherlands, [3]Orthopedics, Erasmus MC, Rotterdam, Netherlands, [4]Molecular Pharmacology, Jichi Medical School, Tochigi, Japan.

TRPV4 is a member of the transient receptor potential (TRP) superfamily and responds to an array of stimuli, including osmolarity, heat, pH, temperature and pressure. Recent findings showing that TRPV4 deficiency leads to reduced sensing of mechanical stimuli led us to explore the role of TRPV4 in bone.
Using real-time PCR, TRPV4 mRNA was abundantly expressed in both osteoblasts and osteoclasts. Femoral cortical and trabecular bone mass (thickness and volume) as assessed by microcomputed tomography was higher in male but not female TRPV4 knockout mice compared to wild type mice.
Next, osteoclast and osteoblast differentiation and function was studied by bone marrow cultures from wildtype and TRPV4 knockout mice. Osteoclast numbers (TRAP staining) as well as the formation of resorption pits (coomassie brilliant blue staining) were significantly reduced in cultures of TRPV4 knockout mice compared to wildtype littermates. In contrast, osteoblast differentiation at day 9 of culture (alkaline phosphatase activity) and matrix mineralization at day 21 (alizarin red) was significantly increased in TRPV4 knockout bone marrow cultures. These data implicate osteoblast-osteoclast uncoupling and support the observed increase in bone mass in male TRPV4 deficient mice.
To assess the possible impact of TRPV4 function on osteoporotic outcome in humans, we extracted data from a recently performed genome-wide association study (Illumina 550K SNP array) within the Rotterdam Study (±7,500 individuals, 55 years and older). Two single nucleotide polymorphisms (SNPs) in the TRPV4 gene showed strong associations with osteoporotic fracture risk (Hazard Ratio 1.79; Confidence Intervals 1.19-2.69), fragility fracture risk (HR 2.58; CI 1.36-4.87) and hip fracture risk (HR 2.75; CI 1.31-5.75) in men, but not in women. This was not affected after adjusting for height, weight, age and bone mineral density (BMD). No associations were found for femoral neck or lumbar spine BMD in either sex.
In conclusion, TRPV4 plays an important role in male but not female bone biology. TPRV4 deficiency results in a male-specific increase in bone mass implicating a negative role for TRPV4 in male bone metabolism. In line with this male specificity, variations in the TRPV4 gene are predicting fracture risk in men but not in women.

Disclosures: ***B.C.J. van der Eerden****, None.*

SA356

See Friday Plenary number F356.

SA357

Disability in Patients with Osteoporotic Pertrocantheric Hip Fracture: A Prospective Study. E. Casado, M. Larrosa*, C. Galisteo*, A. Gómez, P. Lisbona*, E. Graell*, N. Navarro*, M. Moreno*, J. Gratacós*. Rheumatology, Hospital Sabadell, Sabadell, Spain.

Background: Hip fracture in elderly is an important health problem, but the short-medium term disability associated, is not commonly assessed.
Purpose: To study the short-medium term (6 months) disability associated to the stable osteoporotic pertrochanteric hip fracture.
Methods: Prospective study, with inclusion, during a two-year period (March 2002 - December 2003), of all patients older than 65 years with stable osteoporotic pertrocantheric hip fracture (Kyle I-III) who were attended in a 400-bed hospital in Spain. Patients with a moderate-severe disability (impossible to walk by themselves or Barthel <60) prior to hip fracture were excluded. Functional status was evaluated at inclusion (retrospective measurement of disability prior to fracture), and during follow-up (1,3 and 6 months) using: Barthel Index (0-100), the Activities of Daily Living index (ADL, 0-6), and the evaluation of the Walking Ability (WA) by a validated method that gather the need for walking aids.
Results: 82 patients (90% women) with a mean age of 83±7 years were included. The functional status at inclusion was: Barthel index 61±22,6; and 44% and 68% of the patients were independent according to ADL and WA scales respectively (table 1). Disability clearly improved during the follow up, but still remained very important after 6 months (table 1). At six months 37 patients (45%) were lost.
Table:

	Baseline	1 month	3 months	6 months
Barthel Index	88,3±10,8	61±22,6 75	75,9±22,4	85,4±15,7
ADL scale*	44%	3,5%	18%	26,5%
WA scale**	68%	0%	9%	21%

**Independent patients for the 6 basic ADL. **Independent patients for walk*

Conclusion: Hip fracture, even the most stable type, produces an important disability at medium term that is not always taken into account in these patients.

Disclosures: ***E. Casado****, None.*

SA358

See Friday Plenary number F358.

SA359

Family Physician Characteristics that Predict Appropriate X-ray Ordering: Canadian Quality Circle (CQC) National Project. A. Papaioannou[1], G. Ioannidis[1], B. Kvern*[2], A. Hodsman[3], L. Thabane*[1], A. Gafni*[1], A. Walsh*[4], F. Jiwa[5], J. D. Adachi[1]. [1]McMaster University, Hamilton, ON, Canada, [2]University of Manitoba, Winnipeg, MB, Canada, [3]University of Western Ontario, London, ON, Canada, [4]Procter and Gamble Pharmaceuticals, Toronto, ON, Canada, [5]Osteoporosis Canada, Toronto, ON, Canada.

The project is an integrated disease management study designed to improve family physicians' (FPs) adherence with the Osteoporosis Canada 2002 guidelines. This analysis examined the association between appropriate x-ray ordering and FPs characteristics. The guidelines recommend that x-rays should be ordered in all patients with kyphosis or height loss. A total of 225 FPs from across Canada completed the physician characteristics questionnaire, which captured 13 physician characteristics including sex; year of graduation from medical school; country of medical school; does the physician have a full or part-time practice, has hospital privileges, provide house calls, or has after hours call coverage for a defined group of patients; does the physician work in a solo or group practice, work in a teaching practice, is involved with an interdisciplinary team on site, uses electronic health records, or mainly uses fee-for-service billings; and is the physician a current member of the college of family physicians of Canada. During the course of the study, FPs examined a total of 13970 patients who were randomly selected from their practices. The generalized estimating equations (GEE) technique was used to examine the

association between appropriate x-ray ordering and physician characteristics. Odds ratios (OR) and 95% confidence intervals (CI) were calculated. Results indicated that 52% (117/225) of FPs were women, 61% (132/216) provided house calls, 71.5% (156/218) provided after hour call coverage, 30% (67/223) worked in a teaching practice, 32% (73/223) worked within an interdisciplinary team, and 85% (192/225) used fee-for-service billings. Patients were younger in practices of women FPs (56.6% versus 66.4% of patients over the age of 65years) as compared with men FPs. The likelihood of appropriate x-ray ordering was lower if the physician was a woman (OR: 0.76; 95% CI: 0.63, 0.92), provide house calls (OR: 0.71; 95% CI: 0.59, 0.85), worked within an interdisciplinary team (OR: 0.70; 95% CI: 0.56, 0.88), or used fee-for-service billings (OR: 0.66; 95% CI: 0.51 0.85). The likelihood of appropriate x-ray ordering was higher if the physician provided after hour call coverage (OR: 1.87; 95% CI: 1.46, 2.38) or worked in a teaching practice (OR: 1.29; 95% CI: 1.06, 1.58). In conclusion, physician characteristics may influence x-ray ordering and that appropriate x-ray ordering may result in a greater number of vertebral fractures detected.

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SA360

Failure to Perceive Increased Risk of Fracture in Women ≥55 Years. The Global Longitudinal Registry of Osteoporosis in Women. E. S. Siris[1], A. Diez-Perez[2], S. Boonen*[3], C. Cooper[4], A. LaCroix[5], N. B. Watts[6], K. G. Saag[7], S. Silverman[8], R. Dedrick*[9], S. L. Greenspan*[10]. [1]Department of Medicine, Columbia University College of Physicians and Surgeons, New York, NY, USA, [2]Hospital del Mar, Barcelona, Spain, [3]Leuven University Center for Metabolic Bone Diseases, Leuven, Belgium, [4]Southampton General Hospital, Southampton, United Kingdom, [5]Fred Hutchinson Cancer Research Center, Seattle, WA, USA, [6]University of Cincinnati, Cincinnati, OH, USA, [7]University of Alabama-Birmingham, Birmingham, AL, USA, [8]David Geffen School of Medicine, Los Angeles, CA, USA, [9]UMASS Medical School, Worcester, MA, USA, [10]University of Pittsburgh, Pittsburgh, PA, USA.

The purpose of the study was to compare self-perceived risk of osteoporotic fracture among women ≥55 years of age with reported risk factors.
The Global Longitudinal registry of Osteoporosis in Women is an observational follow-up study of women ≥55 years of age recruited by 540 primary physician practices (17 sites, 10 countries). Practices typical of each region were identified by primary care networks organized for administrative, research or educational purposes. All non-institutionalized patients visiting the practice within the prior 2 years were eligible. Self-administered questionnaires were mailed, with 2:1 over-sampling of women ≥65 years of age. Data collected included information on demographics; medical history; risk factors for osteoporosis-related fracture; fracture occurrence; and self-report of prevention, diagnosis and treatment of osteoporosis. Respondents rated their perceived risk of fracture vs women of the same age using a 5-point scale from “much lower” to “much higher.”
Of the women with no risk factors, 90% believed their risk was the same as or lower than that of women of the same age, whereas the majority of women with risk factors failed to appreciate their increased risk of fracture (Table). Among women diagnosed with osteoporosis, 56% believed they were not at increased risk. One quarter of the 15,163 women with a FRACTURE Index ≥5 perceived themselves at higher risk.
Table. Perceived Risk of Fracture Compared with Women of Same Age

		Perceived risk of fracture	
Risk factor	**N**	**Lower**	**Higher**
No risk factor	20,943	41%	10%
History of fracture	11,544	21%	35%
Maternal hip fracture	5972	28%	26%
Parental hip fracture	7397	28%	25%
Weight <125 lb (57 kg)	7705	32%	26%
Smoker	4391	31%	19%
Alcohol >14 units/week	1604	38%	15%
Current steroid use	1484	22%	39%
Rheumatoid arthritis	5196	25%	28%
FRACTURE Index ≥5	15,163	31%	25%
Diagnosis			
Osteoporosis	9353	18%	44%
Osteopenia	8195	27%	25%
Normal	30,166	42%	8%

Most women at elevated likelihood of osteoporotic fracture do not perceive themselves to be at increased risk.

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SA361

See Friday Plenary number F361.

SA362

Risk Factors for Fragility Fracture in a Multiracial Cohort of US Women. The Global Longitudinal Registry of Osteoporosis in Women. S. Silverman[1], J. Adachi[2], S. Gehlbach*[3], S. L. Greenspan*[4], F. Hooven*[3], A. LaCroix[5], R. Lindsay*[6], K. G. Saag*[7], E. S. Siris[8], A. Wyman*[3], N. B. Watts[9]. [1]David Geffen School of Medicine, Los Angeles, CA, USA, [2]St. Joseph's Hospital, Hamilton, ON, Canada, [3]UMASS Medical School, Worcester, MA, USA, [4]University of Pittsburgh, Pittsburgh, PA, USA, [5]Fred Hutchinson Cancer Research Center, Seattle, WA, USA, [6]Helen Hayes Hospital, New York, NY, USA, [7]University of Alabama-Birmingham, Birmingham, AL, USA, [8]Columbia University Medical Center, New York, NY, USA, [9]University of Cincinnati, Cincinnati, OH, USA.

The purpose of the study was to identify risk factors for fragility fracture in a multiracial cohort of women from the USA.
GLOW is a prospective, multinational, observational study. Seventeen sites in 10 countries in North America, Australia and Europe are participating. Practices typical of each region were identified through primary care networks. The present report is limited to data collected from US White, Black and Asian women. All non-institutionalized women ≥55 years of age who visited the practices within the previous 2 years were eligible. Self-administered questionnaires were mailed (2:1 over-sampling of women aged ≥65 years).
In a population of 20,888 women from 6 physician practices, 18,613 were White, 1932 were Black and 343 were Asian. Risk factors for fracture (established from studies of primarily White populations) varied among the 3 groups (Table). White women had the highest prevalence of personal history of fracture, parental fracture, alcohol and steroid use. Black women reported higher rates of smoking, use of arms in rising, rheumatoid arthritis and secondary osteoporosis. Asians had the highest prevalence of weight <125 lb. White women reported higher rates of prior fracture than Black women, which varied from 4.8 times higher for rib to 1.1 times higher for ankle fractures. Both Black and Asian women had hip fracture rates similar to those for White women.
Table. Age-standardized patient risk factors for fracture, by race

Risk factor (%)	White (n=18,613)	Black (n=1932)	Asian (n=343)
Arms used to assist from sitting	35.7	55.3	24.3
Steroid use	34.9	23.5	19.9
Personal history of fracture	24.2	14.4	18.4
Parental fracture	17.5	7.0	11.3
Secondary osteoporosis	16.0	25.3	17.1
Weight <125 lb (57 kg)	15.6	4.7	47.6
Rheumatoid arthritis	8.0	21.4	11.8
Current smoker	7.3	11.5	2.4
Alcoholic drinks (>14 per week)	2.6	0.3	0.3

Racial differences for prevalent fractures and clinical risk factors for fragility fracture were observed in this US cohort. The prevalence of prior fractures among both Black and Asian women, although less than that of White women, is substantial, and places them at risk for future fracture.

Disclosures: ***S. Silverman****, Wyeth, Lilly, Novartis, Alliance 3; Lilly, Novartis, Pfizer, Procter & Gamble 1; Lilly, Argen, Wyeth, Merck, Roche, Novartis 2.*
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SA363

See Friday Plenary number F363.

SA364

Association Between BMD Change, Use of Antiresorptive Agents and Fragility Fracture in Women and Men. C. Berger*[1], L. Langsetmo*[1], L. Joseph*[1], D. A. Hanley[2], K. S. Davison[3], R. Josse[4], N. Kreiger*[4], A. Tenenhouse*[1], D. Goltzman[1]. [1]McGill University, Montreal, QC, Canada, [2]University of Calgary, Calgary, AB, Canada, [3]Laval University, Quebec city, QC, Canada, [4]University of Toronto, Toronto, ON, Canada.

Our objective was to determine the relationship between baseline BMD, longitudinal change in BMD, and fragility fractures in men and women, taking or not taking antiresorptive agents (bisphosphonates, estrogen).
We studied 3635 women and 1417 men aged 50 to 85 years old from the CaMos cohort with at least two BMD measurements in the first five years, with fracture information in the first seven years, and without long-term glucocorticoid use. Multiple logistic regression models were used to assess the relationship between baseline BMD (lumbar spine, femoral neck, total hip and trochanter), BMD change and fragility fractures (any, main, forearm/wrist, ribs and hip) in users and non-users of antiresorptive agents. All models are adjusted for age, height, BMI, previous fracture, diabetes, osteoarthritis and rheumatoid arthritis.
Among antiresorptive non-users, a higher rate of total hip BMD loss is associated with a greater risk of fragility fracture with an odds ratio (OR) of 1.19 (95% CI: 0.94; 1.51) for each 0.01g/cm^2 per year in women, and an OR of 1.60 (95% CI: 1.20; 2.14) for each 0.01g/cm^2 per year in men. Furthermore, the association was even stronger for women with the lowest baseline BMD (first tertile) with an OR of 1.62 (95% CI: 1.12; 2.34) and with the highest loss of BMD with an OR of 1.93 (95% CI: 1.28; 2.90). In men, the association was stronger in those with the lowest baseline BMD with an OR of 1.85 (95% CI: 1.17; 2.93) and in osteopenic men with an OR of 1.73 (95% CI: 1.20; 2.49). In women, the risk of forearm/wrist fragility fracture for a decrease of 0.01g/cm^2 in baseline total hip is 1.04 (95% CI: 1.00; 1.07) in non-users and 1.06 (95% CI: 1.03; 1.08) in users. The risk of forearm/wrist fragility fracture for a decrease of 0.01g/cm^2 per year at the total hip is 1.50 (95% CI: 1.07; 2.11) in non-users compared to 1.03 (95% CI: 0.78; 1.35) in users.
The results show that there is an association between the rapidity of bone loss and fragility fractures in non-users, although stronger in non-user men, suggesting that rate of change is an independent risk factor from baseline BMD. Rapid bone loss is an important risk factor for fragility fractures in subgroups of the population such as those who lose bone faster or those with osteopenia. The inconclusive results between rate of BMD loss and risk of fractures in antiresorptive users support the position that prevention of BMD loss is not the only mechanism for fracture protection with antiresorptives.

***Disclosures:** **C. Berger**, None.*

SA365

See Friday Plenary number F365.

SA366

Positive Celiac Disease Serology and Bone Mineral Density in Adult Women. W. D. Leslie, D. Duerksen*. Department of Medicine, University of Manitoba, Winnipeg, MB, Canada.

Celiac disease (CD) is associated with decreased BMD. Seropositivity with tissue transglutaminase antibody (TTG) and/or endomysial antibody (EMA) is highly specific for CD, while antigliadin antibody (AGA) has lower specificity. We investigated the relationship between BMD and CD serology in a clinical cohort of adult women to determine the role of seropositivity as a risk factor for decreased BMD.
The Manitoba BMD Database (1990-2007) was linked to a provincial database of CD serology (1996-2007) to identify all women in Manitoba over age 20 with BMD results preceding CD serology by 6 m or less (mean interval 3 m). In women with multiple CD serologies only the first was used. CD seropositivity was defined from high specificity assays (CD-HS: TTG or EMA) or low specificity assays (CD-LS: AGA). ANCOVA models (age, height, weight-adjusted) were used to assess the effect of CD seropositivity on BMD at multiple measurement sites (total hip, lumbar spine L1-4, trochanter and femoral neck).
There were 377 matches between the 2 databases (31 CD-HS seropositive, 71 CD-LS seropositive, 296 CD seronegative for both). Mean age was 62 (SD 13) y, and mean Z-scores ranged from -0.30 (SD 1.07) at the femoral neck to -0.86 (SD 1.57) at L1-4. HS-CD seropositivity was associated with lower BMD at all sites, and LS-CD seropositivity was associated with lower BMD at 3 sites (see Table). When HS-CD and LS-CD serology were discordant there was a significant effect of HS-CD seropositivity on BMD (P<.05 for lower BMD at the total hip, trochanter, L1-4) whereas in those with LS-CD seropositivity (with HS-CD negative) BMD was no different from CD seronegative women.
Table: Least-squares mean T-scores (±SE) from ANCOVA models (age, height, weight-adjusted).

	CD-HS Seronegative	CD-HS Seropositive	CD-LS Seronegative	CD-LS Seropositive
Total hip	-1.34 ± 0.06 *	-1.92 ± 0.20	-1.30 ± 0.06 *	-1.65 ± 0.13
L1-4	-1.96 ± 0.07 *	-2.59 ± 0.24	-1.93 ± 0.09	-2.25 ± 0.17
Femoral neck	-1.66 ± 0.05 *	-2.03 ± 0.16	-1.63 ± 0.05 *	-1.88 ± 0.10
Trochanter	-1.62 ± 0.06 *	-2.29 ± 0.19	-1.59 ± 0.06 *	-1.97 ± 0.13

* P<.05, Seronegative vs Seropositive
In conclusion, we found a significantly lower BMD in CD seropositive women compared with seronegative women. This effect was only seen with high specificity CD antibodies (TTG or EMA), and not with low specificity CD antibodies (AGA).

***Disclosures:** **W.D. Leslie**, None.*

SA367

See Friday Plenary number F367.

SA368

Predictors of Osteoporosis Management Among Residents Living in Long-Term Care. L. M. Giangregorio[1], A. Papaioannou[2], J. Hirdes*[3], C. J. Maxwell*[4], M. Jantzi*[3], J. W. Poss*[3]. [1]Kinesiology, University of Waterloo, Waterloo, ON, Canada, [2]Medicine, McMaster University, Hamilton, ON, Canada, [3]Health Studies and Gerontology, University of Waterloo, Waterloo, ON, Canada, [4]Community Health Sciences & Medicine, University of Calgary, Calgary, AB, Canada.

The present study describes the extent to which individuals living in long-term care (LTC) with a recent fracture or diagnosed osteoporosis are receiving osteoporosis management, and identifies factors associated with management.
The sample included 1910 LTC residents living in Ontario and Manitoba, Canada. Data were obtained using the Resident Assessment Instrument Minimum Data Set 2.0. Osteoporosis management was assessed among residents with a diagnosis of osteoporosis or recent fracture. Sociodemographic, clinical and functional characteristics were assessed as correlates of pharmacological osteoporosis management in multivariable logistic regression analyses.
Individuals who had sustained a recent fracture or who had a history of hip fracture or documented osteoporosis represented 27.5% (n = 525) of the sample; use of any osteoporosis medication was reported in 40% of these individuals (from here called the osteoporosis group). Twenty-seven percent (n=140) of residents in the osteoporosis group were documented to be taking both calcium and vitamin D supplementation, 6.5% (n=34) were documented to be taking calcium supplements only, 3.6% (n=19) were taking vitamin D only, and multivitamin use was reported in 19% (n=100) of residents. 54 (10.3%) of residents in the osteoporosis group were taking a bisphosphonate and were not taking vitamin D or a multivitamin. The odds of individuals in the osteoporosis group receiving pharmacological therapy were lower if they had six or more co-morbid conditions, if they used a wheelchair as their primary mode of mobility, if they had depression (DRS 3+), if they had health instability, or if they resided in a facility in Manitoba. Female sex and prescription of ten or more medications were variables positively associated with current use of osteoporosis therapy.
The current study reveals that osteoporosis management is not optimal among individuals at risk of fracture living in LTC facilities, and identifies predictors of osteoporosis management. It may be necessary to incorporate an initiative for health care providers, facility staff, residents and their families to educate them about the importance of fracture prevention strategies in LTC.

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This study received funding from: Ontario Ministry of Health and Long Term Care

SA369

See Friday Plenary number F369.

SA370

The Relationship Between Focal Erosions and Generalized Osteoporosis in Postmenopausal Women with Rheumatoid Arthritis: The Osteoporosis in Rheumatoid Arthritis (OPiRA) Cohort Study. D. H. Solomon[1], J. S. Finkelstein[2], N. Shadick*[1], M. S. LeBoff[3], C. Winalski*[4], M. Stedman*[5], R. Glass*[1], M. A. Brookhart*[5], M. E. Weinblatt*[1], E. M. Gravallese*[6]. [1]Rheumatology, Brigham and Women's Hospital, Boston, MA, USA, [2]Endocrinology, Massachusetts General Hospital, Boston, MA, USA, [3]Endocrinology, Brigham and Women's Hospital, Boston, MA, USA, [4]Radiology, Cleveland Clinic Foundation, Cleveland, OH, USA, [5]Pharmacoepidemiology, Brigham and Women's Hospital, Boston, MA, USA, [6]Rheumatology, University of Massachusetts Medical Center, Worcester, MA, USA.

After 10 years of rheumatoid arthritis (RA), half of all patients have focal erosions and the risk of fracture is doubled. However, little information exists about the relationship between focal erosions and bone mineral density (BMD). We enrolled postmenopausal women with RA who were not current users (but could be past users) of any osteoporosis medications. Participants underwent DXA testing at the hip and spine. A single radiologist blinded to the DXA results and other data read all the hand films and scored them using the Sharp method. We examined the relationship between BMD and erosions using correlation coefficients and adjusted linear regression models.
Sharp scores and DXA results (hip or spine) were examined for 163 women, mean age was 62.4 years (± 9) with an average duration of RA of 13.7 years. Almost all patients were currently using a disease modifying antirheumatic drug. 63% were rheumatoid factor (RF) positive, the median Health Assessment Questionnaire score was 0.7, and the average DAS-28 was 3.8 (± 1.6). The erosion score was significantly correlated with the total hip BMD (Spearman R = -0.33, p < 0.0001) but not with the lumbar spine BMD (Spearman R = -0.09, p = 0.27). Hip BMD was significantly lower in RF positive women (0.83 g/cm^2) versus RF negative (0.89 g/cm^2, p = 0.02). In multivariable models that included age, BMI, and cumulative oral glucocorticoid dosage, neither total hip nor spine BMD were

significantly associated with focal erosions. The relationship between total hip BMD and erosions appears different in patients with: fewer years of disease (<5 versus ≥ 5 years), higher BMI (< 28 versus ≥ 28 kg/m^2), less cumulative oral steroid use (< 960 versus ≥ 960mg), and lower 25-OH vitamin D levels (< 20 versus ≥ 20 ng/dl).
These results suggest that total hip BMD, but not lumbar spine BMD, is associated with focal erosions among postmenopausal women with RA, but this association disappeared after adjustment. Positive RF is associated with a reduced BMD. This study is limited by the fact that most patients had long-standing well-controlled RA. While BMD and erosions may be correlated bone manifestations of RA, their relationship is influenced by other disease-related factors and anatomic sites.

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This study received funding from: NIH-NIA.

SA371

See Friday Plenary number F371.

SA372

Homocysteine Levels in Relation to Physical Performance. N. M. Van Schoor*[1], S. M. F. Pluijm*[2], M. Visser*[1], S. Simsek*[3], Y. Smulders*[3], P. Lips[3]. [1]EMGO Institute, VU University Medical Center, Amsterdam, Netherlands, [2]Public Health, Erasmus MC, Rotterdam, Netherlands, [3]Internal Medicine, VU University Medical Center, Amsterdam, Netherlands.

Elevated homocysteine concentrations are associated with osteoporotic fractures, but the pathofysiologic pathway is not clear. Elevated homocysteine levels might lead to osteoporosis and/or increased fall risk. There is some evidence that elevated homocysteine levels may lead to decreased physical performance. In this study, the relationship between homocysteine levels and physical performance will be examined.
The study was performed using data of the Longitudinal Aging Study Amsterdam, an ongoing multidisciplinary cohort study that started in 1992/93. The current study was performed in persons who participated in the medical interview in 1995/96, and were born in or before 1930 (aged 65 years and older as of January 1, 1996) (n=1509). Data on homocysteine, physical performance and potential confounders were available for 1212 persons. EDTA plasma samples were obtained in the morning, after subjects had eaten a light breakfast. Total homocysteine levels were measured with the use of a fluorescence polarization immunoassay on an IMx analyzer (Abbott Laboratories). Physical performance was assessed in 1995/96 using three tests: the chair stand, the walking test and the tandem stand (total score 0-12). The data were analyzed with homocysteine levels in quartiles (with the lowest quartile as the reference group) using multiple regression analyses.
In total, 589 men and 623 women were included with a mean age of 75.4 years (standard deviation=6.5). No interaction with age was found. The interaction with sex was significant (p=0.076 for the fourth quartile). Therefore, further analyses were stratified on sex. Both in men and women, physical performance decreased with increasing quartiles of total homocysteine in the univariate analyses. After adjustment for age, years of education, region, body mass index, alcohol use, smoking, cognition, heart disease, peripheral arterial disease, stroke, serum 25-hydroxyvitamin D and creatinine, homocysteine was no longer significantly associated with physical performance in men. In women, the second quartile (beta= -0.512, p=0.076) and third quartile (beta= -0.450, p=0.151) were no longer statistically significant. However, the fourth quartile remained significantly associated with decreased physical performance as compared to the lowest quartile (beta= -0.898, p=0.013).
In conclusion, elevated homocysteine levels were associated with decreased physical performance in elderly women.

Disclosures: ***N.M. Van Schoor****, None.*
This study received funding from: Ministry of Health, Welfare, and Sports of The Netherlands.

SA373

See Friday Plenary number F373.

SA374

Risk Factors for Bone Loss in the Hip in 70-Year-Old Women: A Nine-Year Follow-up. G. Sigurdsson[1], S. L. Gudmundsdottir*[1], D. Oskarsdottir*[2], O. S. Indridason*[1], L. Franzson*[3]. [1]Department of Medicine, Landspitali - University Hospital, Reykjavik, Iceland, [2]University of Iceland, Reykjavik, Iceland, [3]Genetic and Molecular Medicine, Landspitali - University Hospital, Reykjavik, Iceland.

The purpose of this study was to examine risk factors for longitudinal bone loss in the hip of 70-year-old women.
Bone mineral density (BMD, g/cm^2) was measured in the femoral neck, trochanter and total hip using dual X-ray absorptiometry (DXA) at five year intervals, in 1997-1998, 2003-2004 and 2007. At each visit, the women also underwent extensive blood test and answered lifestyle questionnaire. Changes in BMD from baseline and its relationship with baseline BMD, weight, lean and fat mass, markers of bone remodeling, hormones, vitamins and lifestyle according to questionnaire as well as weight changes during follow-up were examined. The relationship between 2003-2004 measurements and subsequent bone loss was also studied.
Of 308 women initially studied, 162 returned for the final visit. Mean follow-up time was 9.12 years. Mean 9-year bone loss was -0.83%/year in the femoral neck, -0.47%/year in the trochanter (p<0.01 compared to femoral neck), and -0.53%/year in the total hip (p<0.01/ 0.05 compared to femoral neck/trochanter). Lower baseline (1997) S-25(OH)D correlated with increased bone loss in the femoral neck (r=0.21, p<0.01) and baseline trochanter BMD correlated inversely with trochanter BMD loss (r=-0.41, p<0.01). We found no significant relationship between other baseline measurements and hip BMD change. Based on 2003-2004 measurements, significant risk factors for subsequent bone loss were high serum calcium and low number of leisure walks per week for the femoral neck, lower anabolic index (AI= osteocalcin/crosslaps) and lower estradiol for the trochanter and lower AI for total hip. Bone loss in the femoral neck, trochanter and hip was -9.8%, -7.5% and -8.7% in women experiencing weight loss >5% during follow-up but in women who had stable or increased weight the bone loss was -6.4%, -2.6% and -2.9% respectively (p<0,01 for all sites). Weight loss in the first part of follow-up was not related to bone loss in the latter part of follow up.
This study suggest that high 25(OH)D may reduce longitudinal cortical bone loss. Also, it lends further support to the role of physical activity and weight maintenance as important preventive factors for bone loss in the elderly. Moreover, it shows that the anabolic index, which theoretically gives a ratio of bone formation to bone resorption may predict bone loss and this needs to be studied in more detail.

Disclosures: ***S.L. Gudmundsdottir****, None.*

SA375

Teriparatide Improves Trabecular Architecture as Measured by µCT at Simulated *in vivo* Resolution in the Femoral Neck of Ovariectomized Monkeys. P. Chen*[1], B. Gomberg*[2], M. S. Westmore*[1], K. Krohn[1], M. Sato*[1]. [1]Eli Lilly, Indianapolis, IN, USA, [2]MicroMRI Inc, Philadelphia, PA, USA.

Teriparatide (TPTD) has been shown to improve bone mass, spatial architecture and bone strength in ovariectomized monkeys. Recently, techniques were developed to further characterize architectural features of trabecular bone at in vivo resolution beyond bone volume fraction and conventional histomorphometry. Digital topological analyses (DTA) were developed initially for high-resolution MRI, it is not clear whether it can also be applied to high-resolution images obtained with µCT. We tested the hypothesis that TPTD can improve trabecular bone micro-architecture in the primate femoral neck (FN), as quantified by DTA at simulated in vivo resolution. Ovariectomized monkeys were administered 0 (OVX vehicle, n=20), 1.0 (TPTD1, n=19) or 5.0 µg/kg/day (TPTD5, n=20) TPTD for 18 months. At necropsy, L4-6 and proximal femora were excised and adjacent or contralateral bones were processed for histomorphometry and biomechanical analyses for each monkey. µCT images of the FN were acquired ex vivo at 15 µm isotropic voxel size. Conventional histomorphometry analyses were published previously (Sato et al. 2004). Images were resampled to 75 µm isotropic to simulate in vivo resolution, and then analyzed by DTA, which comprises thresholding, thinning, and topological classification. The analysis identifies surfaces, profiles, curves, and their mutual junctions representing trabecular variations of plates, rods, and their junctions after thinning, and the DTA parameters reflect the micro-architectural integrity of the trabecular network. TPTD significantly improved trabecular architecture measured at in vivo resolution by both histomorphometric and DTA endpoints relative to OVX. Additionally, in vivo resolution DTA endpoints correlated significantly with bone strength in both lumbar vertebra and FN. These results show that TPTD improves the trabecular architecture in the FN of primates using DTA analysis at in vivo data resolution. The data also demonstrate that DTA parameters correlate well with measures of trabecular bone strength at the vertebra and FN. Further clinical studies are needed to confirm the simulated results shown here.

Architecture Parameters†	OVX	TPTD1	TPTD5	Correlation Coefficient	
				Vertebral Strength	Femoral Neck Strength
Histomorphometric parameters					
Trabecular bone volume	0.23±0.06	0.36±0.09*	0.41±0.05*	0.70**	0.64**
Trabecular number	1.43±0.39	2.01±0.50*	2.03±0.42*	0.59**	0.46**
Trabecular separation	0.58±0.18	0.35±0.13*	0.30±0.08*	-0.66**	-0.56**
Trabecular thickness	0.17±0.02	0.18±0.03*	0.21±0.04*	0.26**	0.29**
Topological parameters					
Topological skeleton density	0.11±0.03	0.16±0.04*	0.17±0.03*	0.65**	0.56**
All surface voxel density (10^2)	10.48±2.93	15.44±4.02*	16.82±3.20*	0.65**	0.56**
All curve voxel density (10^3)	1.01±0.98	0.53±0.50	0.42±0.25*	-0.41**	-0.31**
All junction voxel density (10^2)	0.78±0.38	1.81±0.79*	2.39±0.67*	0.67**	0.59**
Surface to curve ratio (10^2)	2.78±4.02	6.25±5.85*	5.73±3.80*	0.52**	0.42**
Topological erosion index	0.32±0.11	0.31±0.06	0.27±0.04*	-0.26**	-0.36**

†Data were expressed as mean ± SD. * P<0.05 compared to OVX, **P<0.05 for correlation coefficients.

Disclosures: ***P. Chen****, Eli Lilly 5.*

SA376

See Friday Plenary number F376.

SA377

Dietary Strontium (Sr) in a Model of Established Osteopenic Rats Reduce the Levels of 25hidroxyvitamin D. M. M. S. Gonzales Chaves*[1], C. Marotte*[1], G. G. Pellegrini*[2], S. M. Friedman*[3], A. Pighin*[4], M. C. de Landeta*[5], S. N. Zeni[1]. [1]Sección Osteopatías Médicas, Hospital de Clínicas, Universidad de Buenos Aires, Buenos Aires, Argentina, [2]Bioquimica General y Bucal, Facultad de Odontologia.UBA, Buenos Aires, Argentina, [3]Bioquimica General y Bucal., Facultad de Odontologia. UBA, Buenos Aires, Argentina, [4]Cs.Basicas. Analitica, Universidad Nacional de Lujan, Buenos Aires, Argentina, [5]Cs.Basicas.Analítica, Universidad Nacional de Lujan, Buenos Aires, Argentina.

Previously we found that vitamin D (vitD) status (i.e.normal or insufficient) interferes with bone mass recovery from bisphosphonate therapy in a model of OVX rats with established osteopenia (Bone 39: 837,2006). Using this model we investigated bone recovery from SrRanelate (SrRa) therapy. After 15 days post surgery and during 45 days, 20 OVX rats fed a diet containing 200IU% D (+D) and 20 a diet lacking vitD (-D) to obtain osteopenic/vitD repleted or depleted groups. Thereafter, 10 -D and 10 +D OVX rats were treated with vehicle (-RaSr) or RaSr (900mg/kg/d) (+RaSr) for 45 days to obtain 4 subgroups (table). A SHAM group was run as control. At 0, 60 and 105 days total skeleton BMD and BMC(teBMC) were assessed by DXA, 25OHD by RIA (Diasorin) and serum Ca and phosphorus (P) by atomic absorption spectrophotometry (AAS) and colorimetric methods, respectively. At day 105, rats were sacrificed and tibia total bone volume (BV/TV) and Sr in bone were determined by histology and AAS, respectively. Results (mean±SE)
Different letter indicates a p<0.05. The were no differences in teBMC, bony Sr and BV/TV between +SrRa treated -D or +D groups. BV/BT in +RaSr was 50% higher than in -SrRa groups but only represented a 10% of SHAM level. The 25OHD levels were significantly lower in +D+RaSr than in +D-RaSr rats. Conclusions: under our experimental conditions Sr may be incorporated into the formed bone independently of vitamin D status. We surprisingly found a diminution in serum 25OHD in +D+SrRa treated rats compared to +D-SrRa groups suggesting, as previously found in chicks (this effect on rats was not previously reported), an inhibition of vitD metabolism to its functional metabolite of nutritional status, 25OHD. (J Biol Chem 247: 5520, 1972).

***Disclosures:* *M.M.S. Gonzales Chaves**, None.*
This study received funding from: Grant PIP 6483, CONICET.

SA378

Aging Dependent and Independent Effects of b2 Adrenergic Receptor Signaling on Bone Formation Induced by Intermittent PTH Treatment in Female Mice. R. Hanyu[1], Y. Saita*[1], J. Nagata[1], Y. Izu[1], H. Hemmi[1], S. Takade[2], Y. Ezura[1], H. Kurosawa*[3], M. Noda[1]. [1]Dept Molecular Pharmacology Medical Research Institute Tokyo Medical and Dental University, Tokyo, Japan, [2]Department of Orthopedics, Tokyo Medical and Dental University, Tokyo, Japan, [3]Department of Orthopedics, Juntendo University school of Medicine, Tokyo, Japan.

Intermittent administration of PTH enhances bone mass levels in postmenopausal osteoporosis patients. This enhancement by PTH on bone mass is modulated by the sympathetic tone through its action on b2 adrenergic receptor. However, whether aging could affect such action of PTH in the presence or absence of sympathetic tone has not been known. We therefore examined the effects of the presence and absence of b2 adrenergic receptor on the anabolic action of intermittent PTH treatment in young adult mice(9w) and compared the results to those in aged adult mice(54w). Cancellous bone mass was enhanced by the treatment with PTH in aged mice, however there was only a trend for the increase in young adult mice. Base line levels of such fraction of bone mass in cancellous bone were about 15% in young while they were about 5% for the aged animal. Interestingly, the enhancement of PTH was approximately 80% in aged mice, but such modulation was only about 30% in young adult mice. In the absence of b2 adrenergic receptor the basal levels of fraction of bone volume were higher than wild type, and in contrast to wild type, PTH no longer enhanced the bone mass levels in both young and aged adult mice. Analysis of the axial bone (vertebrae) also revealed similar trend. However, in this case young adult wild type animals responded to PTH (p< 0.05). Such trend was observed but not statistically significant in aged adult mice. In the absence of b2 adrenergic receptor base line levels were higher than wild type aged mice but such difference was less in young adult mice. Intermittent PTH treatment in the absence of b2 adrenergic receptor was no longer observed in both young and aged adult mice. In contrast to the cancellous bone , cortical bone volume as well as the bone mineral density, which mostly represent the cortical compartment, were similar regardless of the age on the treatment with PTH in the absence or presence of b2 adrenergic receptor. Mean cortical bone thickness was similar. However in terms of cortical bone volume, aged KO mice tended to exhibit higher bone mass levels compared to the young adult KO or WT mice. In conclusion, our data indicatad that there were the age dependent and independent effects of the presence or absence of b2 adrenergic receptor signaling on intermittent PTH treatment for its anabolic action.

***Disclosures:* *R. Hanyu**, None.*

SA379

Prior Treatment with Vitamin K_2 Significantly Improves the Efficacy of Risedronate. Y. Mikuni-Takagaki[1], Y. Matsumoto*[2], Y. Kozai*[2], K. Miyagawa*[1], K. Naruse*[3], H. Wakao*[2], R. Kawamata*[2], I. Kashima*[2], T. Sakurai*[1]. [1]Functional Biologyy, Kanagawa Dental College, Yokosuka, Japan, [2]Maxillofacial Diagnostic Science, Kanagawa Dental College, Yokosuka, Japan, [3]Orthopedic Surgery, Kitasato University School of Medicine, Sagamihara, Japan.

The objective of this study was to determine the best combinatory administration method of bisphosphonate (risedronate) with vitamin K_2 (MK-4) to improve the strength of osteoporotic bone.
Female 9-week ICR mice were given ovariectomy or sham operation. Thirty days later, they were divided into treatment groups (n=8), based on the 16-week daily administration of risedronate (R) and/or MK-4 (K). Ovariectomized (OVX) and sham operated (sham) mice groups and the groups of ovariectomized mice given the treatments were: daily concomitant administration of R and K throughout (2RK), alternate-day administration throughout (R•K•), 8 weeks of R followed by 8 weeks of K (R→K), K followed by R (K→R), or given R and then withdrawn (R→w/drw), K and then withdrawn (K→w/drw), concomitant administration and then withdrawn (RK→w/drw). Mice were terminated after 8- or 16-week treatment. Femurs were subjected to analyses of bone mineral density (BMD) and structure by peripheral quantitative computed tomography (pQCT) and micro focus X-ray tube computed tomography (μCT). Quality of bone was also examined by confocal laser Raman spectroscopic measurements. The serum levels of metabolic markers were measured at 0 (before the treatments), 8, 12, and 16 weeks. Three point bending and compression tests were carried out to assess the mechanical properties of femoral diaphyses and distal metaphyses.
At the 8th week mid point, the trend of improved bone strength was suggested in groups treated with risedronate or MK-4 alone. Quality of bone, judged from the increased matrix-to-mineral ratio and posttranslational processing of collagenous protein, was significantly improved by treating mice with MK-4 alone. Concomitant administration, RK, did not provide equivalent parameters. At the 16th week, cortical BMD in K→R and R→w/drw group femoral diaphyses showed significantly higher values than in the control OVX group. Trabecular structure of K→R and K→w/drw femurs was significantly improved in comparison to the OVX controls. Mechanical tests showed significant improvement of femur strength only in K→R in both diaphyses and metaphyses. Risedronate treatment after MK-4 (K→R) seems to have prevented the CTx rise, which was observed after the withdrawal of MK-4 in K→w/drw.
In conclusion, sequential administration (K→R) was superior to other combinations in treating osteoporotic model mice. Prior treatment with vitamin K_2 significantly improved the efficacy of risedronate by improving the quality of bone matrix.

***Disclosures:* *Y. Mikuni-Takagaki**, None.*

SA380

Withdrawn

SA381

Toremifene Inhibits Osteoclast Activity in vitro and Prevents Orchiectomy-induced Bone Resorption in Male Rats. S. Raghow[1], J. T. Dalton*[1], N. Doyle[2], J. Jolette[2], S. Y. Smith[2], K. A. Veverka[1], R. Narayanan*[1]. [1]Preclinical Research, GTx, Inc, Memphis, TN, USA, [2]Preclinical Research, CRL, Montreal, QC, Canada.

Recent clinical trials in men undergoing androgen deprivation therapy (ADT) for prostate cancer indicated that toremifene (TOR), a SERM, elicited anti-osteoporotic effects. However, the mechanism for this bone protective effect of TOR in males undergoing ADT is not clear. Using orchiectomy-induced bone resorption in adult male rats and rat bone marrow cell culture in the presence of M-CSF and RANKL, we evaluated the mechanism of TOR action in bone. Estradiol was used in vitro for comparison. For the in vivo study, ~9 mo old male rats were randomly assigned to each of five groups containing 10 animals each: baseline, Sham, ORX control and ORX groups treated with TOR at 10 or 30 mg/kg/day. TOR was administered once daily by gavage for 12 months starting one day post-ORX. Biochemical markers of bone resorption (urinary DPD and serum CTx) were evaluated. Cortical (tibia diaphysis) and cancellous (L2 and tibia metaphysis) bone regions were analyzed by histomorphometry. In vitro, TOR at 10^{-6} to 10^{-8} M significantly inhibited osteoclastogenesis (p<0.05 vs. vehicle control) as evidenced by lower number of TRAP-positive multinucleated osteoclasts in bone marrow cultures. In rats, ORX-induced bone turnover as evidenced by increases in CTx and decreases in DPD was suppressed by treatment with TOR at 10 and 30 mg/kg/day. Histomorphometric evaluations of cancellous bone in Sham animals revealed decreases in fractional bone volume (BV/TV) relative to baseline, with further decreases in ORX rats, primarily due to decreases in trabecular number (Tr.N) and separation (Tr.S). These structural changes were associated with marked increases in bone formation rates (BFR) and mineralizing surfaces (MS/BS) consistent with accelerated bone turnover. TOR at 10 and 30 mg/kg consistently reduced Oc.S/BS at both tested sites, demonstrating its bone antiresorptive effects. At the tibia diaphysis, ORX animals showed decreased cortical bone area (Ct.Ar) and width (Ct.Wi) compared to aged Sham males principally due to an increased medullary cavity area (Me.Ar). ORX increased endocortical labeled surface (Ec.L.Pm/Ec.Pm) and BFR but reduced the periosteal labeled surface (Ps.L.Pm/Ps.Pm) significantly (p>0.05). TOR treatment canceled the ORX-induced effects on the periosteal and endocortical surfaces, consistent with reduced bone turnover. In summary, toremifene inhibited

osteoclastogenesis in vitro and showed antiresorptive activity in the bones of orchidectomized male rats, providing a mechanistic basis for its bone sparing effects in men on ADT.

Disclosures: ***S. Raghow****, GTxInc 3.*
This study received funding from: GTx.

SA382

Raloxifene Ameliorates Detrimental Collagen Cross-link Formation in Bone from an Ovariectomized Rabbits With or Without Hyperhomocysteinemia. M. Saito, K. Marumo*, Y. Kida*, C. Ushiku*, S. Soshi*, A. Shinohara*. Orthopaedic Surgery, Jikei University School of Medicine, Tokyo, Japan.

Collagen cross-links are a determinant of bone quality. Cross-links involve two different types; enzymatic divalent immature and trivalent mature cross-links and glycation induced crosslinking (AGEs; pentosidine). We first reported in a human study that hyperhomocysteinemia (HHCY) in patients with an osteoporotic fracture might lead to detrimental crosslinking in bone. Interestingly, homocysteine interferes with normal collagen cross-link formation. Raloxifene (RLX) is thought to ameliorate poor bone quality in osteoporosis. However, there is presently no evidence to demonstrate whether RLX alters the collagen cross-link formation in estrogen-deficiency either with or without HHCY. In order to clarify this issue, we established a rabbit model in which collagen abnormalities were induced by an ovariectomy (OVX) with or without a HHCY and investigated the effects of RLX on collagen cross-link formation in the bone. We divided mature New Zealand white rabbits into the following 6 groups (n=6-9 per group): 1) sham operation, 2) sham+ 1% methionine (Met) diet, 3) OVX only, 4) OVX+ RLX (10mg/kg/day) treated group, 5) OVX+ 1% Met diet, 6) OVX+1% Met diet +RLX. RLX or vehicle was administered by oral gavage for 16 weeks after an initial 16 weeks of OVX or OVX+ 1% Met diet. The femur, vertebra, urine, and blood were collected. We measured cross-link contents in bone by our established HPLC method. Homocysteine levels in the Met diet groups were significantly higher ($p<0.001$) than the normal diet groups. Met diet without OVX resulted in a significant reduction in enzymatic cross-link content (79-85%, $p<0.01$) and a marked increase in the pentosidine content (120-179%, $p<0.05$). The OVX group without the Met diet showed a significant reduction in enzymatic cross-links to the same extent as the Met diet group, whereas the increase in pentosidine was weaker than Met diet group. The OVX+Met diet group showed the most severe detrimental cross-link patterns in the bone. Enzymatic cross-link formation in the OVX +RLX group and the OVX+Met diet +RLX group was restored to the same level as that of the sham group. Pentosidine content in the bone from the RLX treated groups with OVX showed no significant difference in comparison to the sham group. These findings suggest that 1) not only OVX, but also HHCY induced collagen cross-link abnormalitie as well as human osteoporosis, as reported previously, and 2) RLX may increase enzymatic cross-links, while reducing either the glycation or oxidation induced pentosidine in both OVX and HHCY in the OVX rabbit model. In conclusion, RLX may improve bone quality via the alteration of enzymatic and glycation induced cross-links of collagen.

Disclosures: ***M. Saito****, None.*

SA383

See Friday Plenary number F383.

SA384

Effect of Raloxifene and Alfacalcidol on Bone and Joint Pain Assessed by Fall of Skin Impedance (Electroalgometry). T. Fujita[1], M. Ohue*[2], Y. Fujii*[1], Y. Takagi[3]. [1]Medicine, Calcium Research Institute, Kishiwada, Japan, [2]Orthopedic Surgery, Katsuragi Hospital, Kishiwada, Japan, [3]Medicine, National Hospital System Hyogo Chuo Hospital, Sanda, Japan.

By measuring fall of skin impedance in response to exercise loading using an electroalgo - meter (EAM), attempts were made to assess the effect of raloxifen and alfacalcidol on back or knee pain in postmenopausal patients with osteoporosis and/or osteoarthritis against alfacalcidol alone. Subjective pain was simultaneously recorded on a visual rating scale(VRS) dividing the distance between severe and unbearable pain at 100 and no pain at 0. Patients consulting Osteoporosis and Osteoarthritis Clinic of Katsuragi Hospital giving consent to the study after a full explanation on the purpose, method and possible risk of the study were asked to join Group EA to be given 60mg raloxifen and 1μg alfacalcidol or Group A to be given only 1μg alfacalcidol daily in chronological sequence. Exercise loading consisted of standing from a sitting position on a chair and knee bending mainly for knee loading (ST), walking about 30 steps on a flat corridor, climbing up about 10 stairs and down about 10 stairs for combined knee and spine loading (WK), and lying flat on an examining table and standing up again mainly for spine loading(LY). On average of all exercise loading, EAM showed significantly superior analgesic effect in EA than A ($p=0.0239$), but subjective assessment by VRS failed to do so ($p=0.0739$). On standing up, EA significantly more efficiently decreased pain($p=0.0017$) than A according to VRS, but less clear difference was noted by EAM ($p=0.0531$). On WK, on the contrary, significant improvement in EA than A was noted by EAM ($p=0.110$), but only a tendency of improvement was noted on VRS. On climbing up stairs, only EAM revealed a significant improvement in Group EA, but not in A. On LY, only EAM detected significantly better analgesic effect of EA over A ($p=0.048$). Instead of the complete dependance of pain evaluation on subjective judgement, additional assessment by using an objective and quantitative electroalgometry apparently made it possible to analyze the mechanism of pain sensation and action of analgetic agents in more detail. Electroalgometry appearing to be especially sensitive to measure pain on walking confirmed the analgesic effect of raloxifen with alfacalcidol on pain associated with osteoporosis and osteoarthritis.

Disclosures: ***T. Fujita****, None.*

SA385

Bazedoxifene (BZA) in Combinaton with Conjugated Estrogens (CE) Improves Bone Mass and Strength in the Ovariectomized (OVX) Rat. F. Vlasseros*[1], S. Y. Smith*[1], L. Chouinard*[1], R. Samadfam*[1], C. H. Turner*[2], D. Minck*[3], B. Komm[3]. [1]Charles River Laboratories, Montreal, QC, Canada, [2]Indiana University, Indianapolis, IN, USA, [3]Wyeth Research, Collegeville, PA, USA.

Estrogen replacement therapy reduces accelerated bone remodelling, prevents bone loss and increases bone mass (particularly at cancellous bone sites), but is also associated with limiting uterotrophic effects. SERM's bind to estrogen receptors and are intended to prevent OVX-induced bone loss with a favourable influence on lipid profile and an absence of uterotrophic effects. In this study, the combined effects of co-administration of the SERM BZA with CE were evaluated. BZA/CE was administered by oral gavage to OVX Sprague-Dawley rats (24/group) once daily for 52 weeks. BZA was dosed at 0.1, 0.3, or 1.0 mg/kg/day and 2.5 mg/kg/day CE. Additional groups received BZA (0.3 mg/kg/day) or CE alone. OVX and Sham control groups received vehicle.
Clinical serum chemistries were measured and bone turnover was monitored using biochemical markers and dynamic histomorphometry (animals were injected with two fluorochrome labels prior to bone harvesting). Bone densitometry (DXA and pQCT) was measured at the lumbar spine, femur and tibia. Histomorphometry was performed on the tibial diaphysis, L1 and L2 vertebra. Destructive testing was performed by 3-point bending of the femur diaphysis, shearing of the femur neck, and compression of lumbar vertebral specimens. Microscopic evaluations were performed on reproductive tissues.
BZA/CE prevented OVX-induced increases in serum cholesterol and at 0.3/2.5 and 1.0/2.5 mg/kg/day partially inhibited the uterotrophic effect observed with CE alone. At all dose levels BZA/CE decreased bone turnover, completely prevented bone loss due to OVX and preserved bone mass and strength to at least Sham control levels. The high dose combination was associated with increases in biochemical markers of bone formation consistent with preservation of bone mass and slight increases in trabecular bone mass compared to Shams, suggesting this combination may have a superior effect on bone. There were trends that biomechanical competency was also improved at the lumbar spine and femur diaphysis. Overall, protective effects on bone were observed at both appendicular (tibia, femur) and axial (lumbar spine) sites in cancellous and cortical bone. No deleterious microscopic effects (bone quality and/or mineralization) were noted after daily treatment at doses up to BZA/CE 1.0/2.5 mg/kg/day. Combination treatment with BZA and CE may be an effective clinical treatment to prevent the effects of estrogen depletion and maintain bone mass and strength, while reducing serum lipid levels and the uterotrophic effects associated with estrogen treatment alone.

Disclosures: ***B. Komm****, Wyeth Research employee 5.*
This study received funding from: Wyeth Research.

SA386

IC162, a New Flavonoid, Preserves Bone Mass and Microarchitecture without Affecting Uterine Weight in Ovariectomized (OVX) Rats. H. Xie*, E. Liao*. Institute of Endocrinology & Metabolism, The Second Xiangya Hospital of Central South University, Changsha, China.

Flavonoids including genistein have structural similarity to estrogen and have been reported to prevent bone loss caused by estrogen deficiency. IC162 is a new flavonoid compound isolated from vegetable sources. The present study was undertaken to investigate the effects of IC162 on bone and uterus under conditions of estrogen deficiency caused by ovariectomy (OVX). Female Sprague-Dawley rats were randomly divided into six groups: (I) sham operation + vehicle treatment (sham, n = 8); (II) OVX + vehicle treatment (V, n = 9); (III) OVX + 10 μg/kg/d 17β-estradiol treatment (E_2, n = 8); (IV) OVX + 50mg/kg/d genistein treatment (Gen, n = 7); (V) OVX + 33 μg/kg/d IC162 treatment (TL, n = 10); (VI) OVX + 330 μg/kg/d IC162 treatment (TH, n = 8). At 8 months of age, the rats were either sham-operated or OVX, and then treated daily by intraperitoneal injection of vehicle, E_2, genistein, or IC162. After 8 weeks of treatment, bone mineral density (BMD) and bone mineral content (BMC) of the vertebrae and femur were determined by Dual-energy X-ray absorptiometry (DEXA) and microcomputed tomography (μCT), and uterine weight was measured. Vehicle-treated OVX rats showed significant decreases in BMD, BMC, trabecular bone volume (Tb.BVF), trabecular thickness (Tb.Th) and cortical thickness (Cr.Th), and a significant increase in trabecular separation (Tb.Sp) compared to sham controls. These alterations were prevented by treatment of OVX rats with IC162 or E_2. Genistein treatment had the weakest effect on improving these parameters. In addition, treatment with E_2 increased uterine weight to the value of sham controls in OVX rats. Treatment with genistein partially restored uterine weight in OVX rats. However, IC162 had no effect on uterine weight. We conclude that IC162 is efficacious for the preservation of bone mass but does not adversely affect uterine. Hence, our data strongly suggest that IC162 merits investigation as a potential therapeutic alternative to hormone replacement for osteoporosis.

Disclosures: ***H. Xie****, None.*

SA387

A Juvenile Murine Model of Ovariectomy-induced Trabecular and Cortical Osteoporosis. L. Oste*[1], P. L. Salmon[2], G. Kerckhofs*[3], L. Van Rompaey*[1], G. Dixon[1]. [1]Galapagos plc, Mechelen, Belgium, [2]SkyScan N.V., Kontich, Belgium, [3]Department of Metallurgy and Materials Engineering, Catholic University of Leuven, Leuven, Belgium.

Introduction. The ovariectomised mouse is routinely used as a model of osteoporosis, and commonly a mature adult age such as 4 months at surgery is used. Here we validate a murine ovariectomy model in young (6 week) growing mice in three strains, evaluating trabecular and cortical osteopenia by micro-computed tomography (micro-CT) bone morphometry.
Materials and Methods. Mice from the BALBc, C3H and C57BL/6 strains were either ovariectomised or sham operated at 6 weeks age, and femurs and tibias were harvested from them five weeks later. Micro-CT morphometric analysis of these bones was carried out on the distal femur and proximal tibia.
Results. All three strains showed substantial trabecular bone loss due to ovariectomy, slightly less in BALBc than in the C3H or C57BL/6 strains. Architectural parameters such as Trabecular pattern factor and fractal dimension showed profound structural change in the C3H and C57BL/6 strains but less change in BALBc. All three strains showed significant cortical osteopenia in the ovariectomised groups but with a different pattern between strains: while the cortex expanded in BALBc and C3H mice both periosteally and endosteally, in the C57BL/6 mice the periosteum contracted slightly. Cortical porosity showed a large ovariectomy-related increase in the BALBc and C3H strains but less change in C57BL/6.
Conclusions. Comparing the results to published data for the more mature mouse OVX model, OVX-related osteopenia in both trabecular and cortical bone is much more pronounced and significant in the younger mice. Older mouse bone morphometry is compromised by sparse trabecular bone. The growing mouse OVX model is justified by evidence that the changes seen are directly linked to withdrawal of estrogen, not an epiphenomenon of growth, and that, architecturally, these changes are generally similar in young and old age. Specific cortical OVX-related changes namely thinning, periosteal and endosteal expansion, endocortical trabecularisation and increased porosity, all associated with human perimenopausal femoral osteoporosis, were all seen in this growing mouse OVX model. Thus a growing mouse OVX model may be useful in studying effects of estrogen on bone throughout female adulthood.

***Disclosures:* P.L. Salmon**, *SkyScan N.V. 3.*
This study received funding from: SkyScan.

SA388

Skeletal Effects of a Fibroblast Growth Factor Agonist (F2A) in Ovariectomized Rats. S. E. Franz*[1], M. K. Altman*[1], J. N. Stabley*[2], J. I. Aguirre[1], M. E. Leal[1], X. Lin*[3], P. O. Zamora*[3], T. J. Wronski[1]. [1]Physiological Sciences, University of Florida, Gainesville, FL, USA, [2]Applied Physiology and Kinesiology, University of Florida, Gainesville, FL, USA, [3]BioSurface Engineering Technologies, Inc, Rockville, MD, USA.

F2A is a synthetic peptide designed as an agonist for FGF2. It binds with high affinity to FGF receptor 1. A previous study showed that F2A, when applied locally, accelerated bone repair in a rabbit experimental model of tibial fractures. The purpose of the current study was to determine if systemically-administered F2A had skeletal effects in ovariectomized (OVX) rats and adverse side effects in non-skeletal tissues. Groups of sham-operated and OVX rats were maintained untreated for six weeks postovariectomy to allow for cancellous bone loss to occur in the latter animals. These groups (N=6) were then treated IP with vehicle or varying doses of F2A (0.3, 1.0, or 3.0 mg/kg) for 14 consecutive days. All rats were injected SC with declomycin and calcein 10 and 3 days prior to necropsy, respectively, in order to measure surface-referent, bone formation rate. At necropsy, hematocrit was found to be normal in all F2A-treated OVX rats. Histological samples of the liver, kidney, lung, and heart showed no significant abnormalities in these animals. The proximal tibial metaphyses of vehicle-treated OVX rats were characterized by cancellous osteopenia and increased bone turnover compared with vehicle-treated control rats. F2A did not increase tibial cancellous bone or osteoid volume at any dose tested, but OVX rats treated with 1 mg/kg did exhibit a significant 25% increase in osteoclast surface, an index for bone resorption. Normal fluorochrome labeling of bone occurred, which indicates that mineralization of osteoid was not impaired by F2A, as was previously observed in FGF2-treated OVX rats. Furthermore, all 3 doses of F2A induced a modest increase in cancellous bone formation rate. In contrast, periosteal bone formation in the tibial diaphysis was unaffected by F2A treatment. Based on our past studies with systemically-administered FGF2, the skeletal effects of F2A are more subtle than those of FGF2. F2A appears to induce a modest increase in both bone resorption and formation, but these skeletal processes are in balance so that cancellous bone mass is not increased in F2A-treated OVX rats. However, F2A did not induce the anemia and impaired bone mineralization seen with FGF2. The increase in cancellous bone formation and the lack of side effects is supportive of F2A for local bone fracture applications. Therefore, locally-delivered F2A appears to have a favorable pharmacokinetic profile, and yet has minimal side effects even if the peptide circulates systemically.

***Disclosures:* S.E. Franz**, *None.*
This study received funding from: Biosurface Engineering Technologies, Inc.

SA389

See Friday Plenary number F389.

SA390

The Use of Alternative Medicine for Treating Osteoporosis in Korea. S. Han*, J. Park*, Y. Park*, E. Bae*, Y. Kim*. Department of Family Medicine, Dong-A University Hospital, Pusan, Republic of Korea.

As life expectancy increases, the interest in osteoporosis is now an international trend. People in Korea are becoming more and more interested in osteoporosis, but many patients still prefer alternative medicines such as health foods than going to the hospital. The authors of this study aimed at surveying what the Korean patients select as a treatment for osteoporosis and wanted to offer the information to doctors.
The study took place in 3 cities of Korea from June, 2007 to December, 2007. The objectives were 1,524 randomly selected postmenopause women between the age of 50 and 70, and a questionnaire was performed.
74% of the objectives had bone mineral densitiometry(BMD) tests. 36% of them were diagnosed with osteoporosis, and 64% had been diagnosed with osteopenia.
Among them, 83% had experienced one or more alternative medicine such as health foods. Safflower seed(64%), glucosamine(43%), calcium complex food(41%), chlorella(29%), vitamin D enriched food(25%), herb medicine(21%), ginseng steamed red(19%), plant estrogen(18%), soybean protein food(15%), acupuncture(6%), physical therapy such as cupping(5%)were included in the descending order.
The mean term they took the medications was 7.5 months.
The satisfaction on the alternative medicines was the highest in safflower seed (53%), and calcium complex food(42%), ginseng steamed red(41%), plant estrogen(35%), herb medicine(28%) followed. The satisfaction on alternative medicine itself (42%) was slightly higher than treatment from doctors(38%).
Many patients use alternative medicine for the treatment of osteoporosis, and they are highly satisfied with it. It would be very helpful if the doctors take interest in alternative medicine in treating osteoporosis and provide the patients with accurate information.

***Disclosures:* S. Han**, *None.*

SA391

See Friday Plenary number F391.

SA392

Strontium Ranelate and Teriparatid (PTH 1-34) Enhance Fracture Healing in Osteoporotic Sprague Dawley - Rats. B. Habermann*[1], G. Olender*[2], C. Eberhardt*[1], P. Augat*[2], A. A. Kurth*[1]. [1]Orthopedic Hospital Frankfurt, Frankfurt, Germany, [2]Biomechanical Research Laboratory Murnau, Murnau, Germany.

Strontium ranelate and PTH 1-34 both result in an increased formation of bone when given to patients with osteoporosis. The purpose of the study was to elaborate if strontium ranelate and PTH 1-34 affect fracture healing when given the first time after an osteoporotic fracture and if they show any benefit in stability of the callus.
45 Sprague Dawley rats were ovariectomized at the age of 12 weeks. After 12 weeks, an osteopenia was diagnosed using Dual x-ray energy (DXA). Then, a standardized closed mid-diaphyseal fracture of the femur stabilized by a K.-wire was produced. Afterwards, the animals were randomly put into three groups. The first group was substituted with 0,9% NaCl s.c., the second received 20 µg PTH 1-34 3x/week s.c. and the last group was treated with 600 mg strontium ranelate per kg body weight orally. After 28 days the bones were excised. The samples were subsequently scanned using MicroCT and, afterwards, torsion testing on the bones was done.
Strontium ranelate and PTH 1-34 both showed a significant increase in bone volume of the callus. (strontium ranelate +46.4%, $p<0,05$; PTH 1-34 +31.9%, $p<0,05$) though difference among them was not significant. This was also expressed in callus tissue volume (strontium ranelate +32.4%, $p<0,05$, PTH 1-34 +6%, $p>0,05$) while the increase under substitution with strontium was significantly higher (+ 24.8%, $p<0,05$). Torsion to bone fracture was significant higher under substitution with strontium ranelate while substitution with PTH 1-34 showed no difference.
Strontium ranelate and PTH 1-34 are both considered to be anabolic when given to patients with osteoporosis. Intermittend substitution of PTH 1-34 causes an increase in bone mineral density by an enhancement of osteoblast proliferation. Strontium ranelate does not only increase collagen synthesis and enhance the activity of osteoblasts by activating the calcium sensing receptor but also causes a downregulation of osteoclast acitivity by reducing preostecoclast differentiation. There is evidence that it also modifies the OPG/ RANK(L) system.
Our results support that both strontium ranelate and PTH 1-34 may enhance fracture healing with an increase in callus tissue and bone volume. Callus in strontium substituted animals seems to be more resistant to torsion in comparison to sham-treated animals or animals being treated with PTH 1-34. Strontium had significant better results in torsion testing to the fracture point. This may be an effect from a higher callus volume and callus bone volume / tissue volume.
The animal experiments were approved by the Regierungspraesidium Darmstadt, Germany (F-119/04). This study was supported by the Elsbeth Bonhoff Stiftung.

***Disclosures:* B. Habermann**, *None.*
This study received funding from: Elsbeth Bonhoff Stiftung.

SA393

QUS, BMD and QCT in Women with Established Osteoporosis Treated with Teriparatide. C. Cepollaro[1], R. Monaco*[1], F. Cioppi*[1], L. Masi[1], A. Falchetti[1], G. Marcucci*[1], G. Caracchini*[2], G. D'Elia*[2], A. Tanini*[1], M. Brandi[1]. [1]Department of Internal Medicine, University of Florence, Florence, Italy, [2]SOD Radiodiagnostica 1 CTO, Azienda Ospedaliero-Universitaria Careggi, Florence, Italy.

This study was aimed to evaluate the effect of a 18-month teriparatide treatment on bone mineral density (BMD), quantitative ultrasound (QUS) and quantitative computed tomography (QCT) in women with established osteoporosis. We studies 31 women with established osteoporosis previously treated with antiresorptive drugs. They were given teriparatide (Forsteo, Eli Lilly) by subcutaneous injection, at the dose of 20 µg once daily, plus calcium and Vitamin D. In all subjects BMD at lumbar spine (BMD-LS), femoral neck (BMD-FN) and total femur BMD (BMD-T) was assessed at baseline, and every 6 months for 18 months. At the same time we also performed QUS at phalanxes, by Bone Profiler-IGEA (amplitude dependent speed of sound: AD-SoS, ultrasound bone profile index: UBPI, bone trasmission time: BTT). At baseline and after 18 months vertebral QCT scans of the first, second and third lumbar vertebrae were obtained using a specific software (OSTEO) to quantify the volumetric mineral density (vBMD) of somatic trabecular bone.
Teriparatide significantly ($p<0.001$) increased BMD-LS; no significant variation in femoral subregions was found during the study period even though BMD-T tended to increase with the duration of PTH treatment and BMD-FN showed a reduction at month 6 with a progressively return to basal values. No significant changes were observed also for UBPI and AD-SoS, even if the last decreased at month 6 and thereafter it tended to increase. BTT significantly decreased at all time points ($p<0.05$). Teriparatide induced an important and significant ($p<0.001$) increase in vBMD at lumbar spine, as assessed by QCT. Our study shows that in women with established osteoporosis teriparatide determined the expected increase in BMD and vBMD at lumbar spine, with a less strong or negative effect at skeletal sites mainly composed by cortical bone. Further studies in larger population would be necessary to define the clinical role of QUS parameters in patients treated with teriparatide.

***Disclosures:** **C. Cepollaro**, None.*

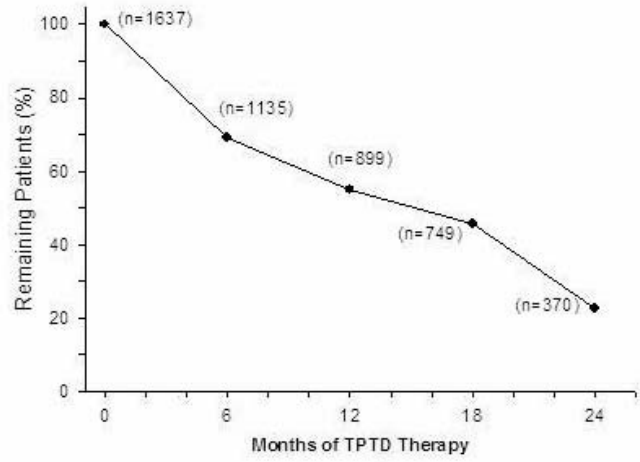
SA394

See Friday Plenary number F394.

SA395

Teriparatide Therapy in a Community Setting: Persistence and Use of Other Osteoporosis Medications in DANCE. K. Taylor[1], D. T. Gold[2], P. Miller[3], P. Chen*[1], M. Wong[1], K. Krohn[1]. [1]Eli Lilly, Indianapolis, IN, USA, [2]Duke University, Durham, NC, USA, [3]Colorado Center for Bone Research, Lakewood, CO, USA.

The Direct Assessment of Non-vertebral Fracture in Community Experience (DANCE) observational study completed enrollment of 4106 patients on Dec 3 2007. Study investigators prescribe TPTD 20 µg/d for up to 24 months to patients, and follow them for another 24 months. As this cohort matures, more robust "real world" observations may be derived from patients' experience with teriparatide (TPTD) in a community setting. Poor persistence to osteoporosis therapy can reduce therapeutic benefit, despite the fracture risk reductions shown in clinical trials [Mayo Clin Proc 2006;81:1013-22]. Overall 1-yr persistence with osteoporosis therapies was found to be between 25-45% [Maturitas 2004;48:271-87, Arch Int Med 2005;165:2414-9]. This updated analysis from DANCE shows the persistency to therapy in 1637 patients with data on their first and last dose of TPTD (Figure). The percentage of patients remaining at 6, 12, 18, and 24 months was 69%, 55%, 45%, and 23%, respectively.
Of the 256 patients (16%) who reported concomitant use of ≥1 other bone-active agent during the TPTD treatment phase, 214 (84%) had initiated these other agents prior to starting TPTD. Estrogen was most often used (n=111), followed by alendronate (n=57), raloxifene (n=45), risedronate (n=32), and calcitonin (n=25). After cessation of TPTD therapy, 747 patients (45.6%) reported using ≥1 other bone-active agent in the follow-up phase, and of these patients, 220 reported having used ≥1 other bone-active agent concomitantly during the TPTD treatment phase. Bisphosphonates, such as alendronate (n=244), ibandronate (n=226), risedronate (n=152), and zoledronic acid (n=23), were most commonly used in the follow-up phase. Estrogen (n=104) and raloxifene (n=76) were also used. In summary, the present analysis shows that 1) about half of patients who started TPTD persist with therapy at 1 year; 2) patients who took other bone-active agents concomitantly with TPTD had initiated use of these agents prior to starting TPTD; and 3) about half of patients take other bone-active agents after stopping TPTD therapy. Continued analysis of the DANCE cohort may provide insight on factors affecting persistence to TPTD therapy, on how TPTD is used among a spectrum of osteoporosis medications, and offer new approaches to optimize TPTD use in the community.



***Disclosures:** **K. Taylor**, Eli Lilly 5.*
This study received funding from: Eli Lilly and Company.

SA396

See Friday Plenary number F396.

SA397

Repetitive Rapid Delivery of Pharmacologically-Active hPTH 1-34 Across Human Skin Without Injection. S. Ish Shalom[1], Y. Kenan*[2], T. Matsumoto*[3], R. Neer[4]. [1]Metabolic Bone Diseases Unit, Rambam Medical Center, Haifa, Israel, [2]TransPharma Medical Ltd, Lod, Israel, [3]Department of Medicine and Bioregulatory Sciences, University of Tokushima Graduate School of Medical Sciences, Tokushima, Japan, [4]Endocrine Unit, Massachusetts General Hospital, Boston, MA, USA.

TransPharma has developed a system for transdermal delivery of hPTH 1-34 (TD) to alleviate the discomfort of injections (SC) and improve patient acceptance of and compliance with hPTH 1-34 therapy. The system utilizes RF ablation to create MicroChannels in the upper skin, allowing rapid diffusion of peptide from a subsequently-applied drug patch into the inner skin and systemic circulation. We compared the safety, tolerability, pharmacokinetics (PK), and type I procollagen N-terminal propeptide (PINP) based pharmacodynamic (PD) profile of TD vs. SC hPTH 1-34 administered once-daily for 7 days. 48 healthy post-menopausal women, age 65 ± 4 years, were randomly allocated in 3 blocks of 16 to a daily TD dose of 50, 70, or 90 µg or a daily SC dose of 20 µg (FORTEO®). On days 1 and 7 we measured fasting serum PINP 1 hour before dosing (after which participants ate breakfast), and measured serum hPTH 1-34 15 minutes before and 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 14 and 24 hours after TD or SC administration of the peptide. To minimize assay-related bias, all sera for a woman were measured in the same hPTH 1-34 or PINP assay. In each treatment group the mean PK profile on day 7 (presented in the Table below) did not differ significantly from that on day 1, indicating reproducible peptide delivery and no peptide accumulation.

Treatment	Cmax (pg/ml) mean ± SD	Tmax (h) mean ± SD (range)	AUC_{0-t} (hr*pg/ml) mean ± SD	P1NP absolute change from baseline (µg/L) (% Change) mean ± SD
TD 50 µg (n = 12)	53 ± 16	2.0 ± 0.5 (1.5-2.5)	135 ± 48	13 ± 7 (28 ± 15)
TD 70 µg (n = 12)	68 ± 28	2.3 ± 0.3 (1.5-2.5)	162 ± 73	13 ± 9 (26 ± 15)
TD 90 µg (n = 12)	90 ± 37	2.5 ± 0.6 (1.5-4.0)	250 ± 116	12 ± 9 (22 ± 15)
SC 20 µg (n= 10-12)	72 ± 21	0.6 ± 0.4 (0.25-1.0)	117 ± 45	8 ± 17 (20 ± 29)

Cmax occurred approximately 2 hours after TD application and 0.6 h after SC injection. Serum hPTH 1-34 was quantifiable 1.5-3.5 hours longer after TD dosing than after 20µg SC dosing. The bioavailability of TD hPTH 1-34, calculated from the area under the time-concentration curves, was ~40% of SC injection. Following 6 days of hPTH 1-34 treatment, serum PINP increased significantly in each treatment group by paired t-test (p*< 0.0001- 0.05). Transdermal therapy was well-tolerated; application sites showed only very minor and transient erythema and edema, and no infections. These safety, tolerability, PK and early PD data demonstrate that TD hPTH 1-34 is a promising alternative to the currently-marketed injections.
* P values are based on percent changes

***Disclosures:** **S. Ish Shalom**, TransPharma Medical Ltd. 2.*
This study received funding from: TransPharma Medical Ltd.

SA398

See Friday Plenary number F398.

SA399

A Phase 1, Randomized, Double-Blind, Placebo Controlled Trial of the PK/PD Effect and Safety of ZT-031, a hPTH Analog, in Healthy Elderly Women. C. L. Barclay*, R. Anderson*, D. Krause, B. MacDonald. Zelos Therapeutics, Inc, West Conshohocken, PA, USA.

Parathyroid hormone (PTH) mediates its anabolic effect on bone via transient stimulation of PTH receptors (PTHr) on osteoblasts, leading to adenylyl cyclase (AC) activation, cyclic AMP production and increased bone formation. AC activation in renal tubular cells results in renal Ca retention and increased PO4 excretion. ZT-031 is a novel, 31 a cyclic analog of human PTH, currently in late clinical development for the treatment of post-menopausal osteoporosis.
The purpose of this study was to evaluate the safety, PK, and PD effect of ZT-031 in healthy elderly women.
20 healthy adult women ≥ 65 years of age (mean 69 y) were randomized to 7 days of treatment with 20, 30, or 40 µg of ZT-031 or placebo, given by daily SC injection. PD markers included urine cAMP on Days (D) 1 and 7 pre- and 2h post-dose. PK parameters were evaluated using a validated ELISA. Safety parameters included adverse events (AEs), EKGs and serum Ca as well as urinary Ca and PO4.
Urine cAMP increased in dose-proportional fashion 2h after dosing on D1 and 7; increases are shown in the Table.

Dose	20 µg n=7	30 µg n=4	40 µg n=6	Placebo n=3
Day 1 Urine cAMP[1,2]	542.7	728.4	1941.3	-15.2
Day 7 Urine cAMP[1,2]	394.3	1026.0	1375.8	28.7

[1] µmol/mmol Cr [2] $p < 0.01$

By 8 h post-dose, mean urine cAMP returned to baseline (BL) for all groups. Subsequent to the increase in urine cAMP a sequence of reduced urinary Ca and increased urinary PO4 excretion followed by the converse changes in serum were observed. All changes in Ca and PO4 profile returned to BL by 8-12 h post dose. PK parameters were similar on Days 1 and 7. Cmax and AUC(0-4h) were approximately dose proportional whereas T1/2 (average 1.12h for all time points/doses), clearance and volume of distribution were dose-independent. A total of 72 AEs was reported by 17 subjects reported; none was severe or serious. The frequency of AEs did not vary by dose. Headache was the most common AE. Three Ca values > ULN (2.55 mmol/L) were observed, 1 in a 30 µg subject and 2 in 40 µg group. None was symptomatic, persistent or exceeded 2.61 mmol/L. No subject withdrew due to a treatment-emergent AE. A dose-dependent effect on resting heart rate was observed.
ZT-031 was well tolerated and exhibited dose proportional PD and PK responses in healthy elderly women. The effect on serum and urinary Ca/PO4 profiles indicated that cAMP generation was secondary to activation of PTHr. The observed PK/PD profiles are appropriate for the intermittent stimulation of PTHr required for the anabolic effects of a PTH analog.

Disclosures: ***D. Krause****, Employee of Zelos Therapeutics 5.*
This study received funding from: Zelos Therapeutics, Inc.

SA400

Bone Mineral Density and Vertebral Fracture in Women with Breast Cancer and Antiaromatase Therapy. E. Hoppe*[1], B. Bouvard*[1], C. Lassalle*[1], R. Levasseur[1], M. Audran[1], D. Chappard[2], E. Legrand[1]. [1]Rheumatology and Inserm U 922, Centre Hospitalier Universitaire, Angers, France, [2]Inserm U 922, Centre Hospitalier Universitaire, Angers, France.

Background: Recovery from breast cancer in women has been achieved using chemotherapy, radiotherapy that can affect bone tissue. Furthermore, aromatase inhibitors therapy can accelerate bone loss and increase the fracture risk.
Objectives: Prospective and longitudinal study to evaluate bone mineral density and fractures in 515 women with treated breast cancer, before and after one year of aromatase inhibitors therapy.
Methods:. Medical history, clinical risk factors for osteoporosis, non spine fractures, hip and spine Bone Mineral Density (BMD) were checked at baseline and after one year of antiaromatase therapy. Bone remodelling was evaluated by serum levels of C-telopeptide (CTX), osteocalcin and bone specific alkalin phosphatase (BSAP). Spinal X-ray films were analyzed independently by two trained investigators who were unaware of the patient status. Vertebral fracture was defined as a reduction of at least 20 percent in the anterior, middle or posterior vertebral height
Results: At baseline, 44.2% of women had a BMD T-score <-1 (on spine or hip) and 21.6% had a T-score <-2. Spinal radiographs revealed that 21.7% women had at least one vertebral fracture and 13 (6.8%) had 2 or more vertebral fracture. 13.7% of women reported non spine fractures. Logistic regression analysis showed that age (+5 years : OR=1.46, CI 95% 1.15-1.85), weight (-1 kg : OR =1.14, CI 95% 1.08-1.19), self-reported non spine fracture (OR = 4.17, CI 95% 1.80-9.63) and radiographic vertebral fractures (OR = 6.12, CI 95% 2.42-15.71) were associated with the presence of low BMD (T-score <-2 on spine or hip). Women with osteoporosis (25% of the initial cohort) received risedronate (35 mg weekly) and were investigated after one year of antiaromatase therapy : the mean BMD was increased by 1.77% on spine and by 0.78% on hip. We observed that 5.4% of these women have a new fracture but only 3.6% had a new vertebral fracture.
Women with normal BMD or osteopenia (75% of the initial cohort) didn't received any anti osteoporotic therapy and were investigated after one year: the mean BMD was reduced by 1.25% on spine and by 1.75% on hip. We observed that 5.8% of these women have a new fracture but only 1.5% had a new vertebral fracture.
Conclusion: These results strongly suggest that (1) 20 to 25% of women with treated breast cancer have low BMD or osteoporotic fractures before starting antiaromatase therapy (2) these women should benefit from clinical evaluation, BMD measurement and specific bone therapy, (3) in women with normal BMD or osteopenia, the rate of bone loss and the incidence of fractures (after one year) is quite similar that expected for postmenopausal women.

Disclosures: ***E. Legrand****, None.*

SA401

See Friday Plenary number F401.

SA402

Renal Safety Across a Wide Range of Dosing Regimens of Risedronate. P. Miller[1], R. Miday*[2], A. Klemes[2], D. Ramsey*[2]. [1]Colorado Center for Bone Research, Lakewood, CO, USA, [2]Procter & Gamble Pharmaceuticals, Mason, OH, USA.

The incidence of both osteoporosis and renal insufficiency increases with age; thus, the effect of osteoporosis treatments on renal function is a clinical concern. This retrospective analysis was conducted to study the influence of multiple dosing regimens of risedronate on the renal function of postmenopausal women with osteoporosis or osteoarthritis.
Combined data from over 14,000 patients in the Risedronate phase III clinical trials of post-menopausal osteoporosis (PMO) and osteoarthritis were analyzed across multiple dose regimens; 5 and 15 mg daily, 35 and 50 mg weekly, 75 mg on 2 consecutive days monthly or 150 mg monthly, or placebo. Median treatment duration was 2 years. Subgroups with baseline renal function impairment or renal risk factors were also examined. Serum creatinine (SCr), estimated serum creatinine clearance (CrCl - Cockroft-Gault) and renal function related adverse events (AEs) were analyzed.
94% of subjects were female, with a mean age of 70 years. The frequency distributions of changes in CrCl from baseline to endpoint value were not different between placebo and risedronate groups or between different dose/regimen groups, for each of the populations examined. Also, there was a similar frequency of patients who developed abnormal SCr or who developed SCr increases > 0.5 mg/ml from baseline, within each of the active treatment group and placebo comparisons (Table 1). Patients with baseline renal impairment (CrCl ≤ 50 ml/min) or baseline renal risk factors (diabetes mellitus, hypertension, ACE inhibitor or NSAID treatment) also did not show treatment group differences. Renal function-related adverse events were not different across groups, all ≤ 2%. While risedronate serum Cmax levels for 75 mg and 150 mg are approximately 11 and 29-fold greater than for 5 mg, there is no indication of adverse renal effects from these higher doses.
Risedronate demonstrates a favorable renal safety profile across a wide range of doses, regimens and patient populations with no evidence of effect on renal function parameters.

Table 1
Renal Safety Endpoints in Risedronate Post-Menopausal Osteoporosis Studies

Parameter	5 mg Daily		75 mg Two Consecutive Days (Monthly)		150 mg Once a Month	
	Placebo %	5 mg Daily %	5 mg Daily %	75 mg/2cd %	5 mg Daily %	150 mg Monthly %
Serum Cr - Treatment Emergent Abnormal[1]	8.0	8.4	1.7	1.4	1.7	0.8
Serum Cr - ? from Baseline > 0.5 mg/dL at Endpoint	0.7	0.7	0.3	0.2	0.0	0.3
Renal Function Adverse Events	1.9	1.5	1.1	0.6	0.5	0.5
	Mean(SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
CrCl - ? from Baseline to Endpoint	-0.62 (10.14)	-0.42 (9.56)	-3.62 (11.13)	-4.12 (10.94)	-1.14 (7.59)	-1.45 (8.06)
CrCl - ? from Baseline to Endpoint (Baseline CRCL ?50)	0.95 (6.36)	0.85 (6.40)	0.97 (8.51)	-0.26 (5.44)	-0.35 (4.79)	0.73 (5.96)

[1]Treatment emergent abnormal serum creatinine are patients with a normal baseline creatinine and 1 or more abnormal values after dosing. Number of patients varies depending on analysis but numbers of patients treated are the following: 5 mg Daily Studies (4878 placebo and 4846 5 mg daily), Two Consecutive Days (613 5 mg daily and 616 75 mg/2CD), and Once a Month (642 5 mg daily and 650 150 mg monthly).

Disclosures: ***P. Miller****, P&G 2.*
This study received funding from: The Alliance for Better Bone Health (Procter & Gamble Pharmaceuticals and sanofi-aventis).

SA403

See Friday Plenary number F403.

SA404

Fracture Reduction During Two Years of Treatment with Risedronate or Alendronate, a Retrospective Cohort Study. P. Delmas[1], J. Lange[2], S. Silverman[3], N. Watts[4], R. Lindsay[5]. [1]INSERM Research Unit 831, Lyon, France, [2]P&G Pharmaceuticals, Mason, OH, USA, [3]Cedars Sinai Medical Center, Los Angeles, CA, USA, [4]Bone Health and Osteoporosis Center, Cincinnati, OH, USA, [5]Helen Hayes Hospital, West Haverstraw, NY, USA.

In the published REAL cohort study,[1] we observed in the first year of treatment that patients using risedronate had a lower incidence of hip and nonvertebral fractures than patients using alendronate. We now have additional data that extends these observations into a second year of therapy. For the current analysis, we used patients with a single bisphosphonate prescription as a referent cohort.
The original study population of women over age 65 included new users of once-a-week dosing of risedronate (n = 12,215) or alendronate (n = 21,615) who initiated treatment between July 2002 and June 2004 within records of health services utilization. This population is followed through June 2006 or until no longer therapy adherent. As a referent cohort, we selected patients who filled only a single prescription of alendronate or risedronate during the observation period (n = 5,390). Proportional hazard modeling was used to compare the incidence of hip and nonvertebral fractures between cohorts, adjusting for potential differences in baseline fracture risk.
Based on history of clinical fractures and age upon study entry, the cohort at highest baseline fracture risk was the referent cohort, followed by the risedronate cohort, then the alendronate cohort. At the end of two years of observation for the referent cohort, the cumulative incidence of hip fractures was 1.9% and of nonvertebral fractures was 6.3%. Compared to the referent cohort, patients on risedronate had an approximately 40% lower incidence of hip fractures at 6, 12, 18, and 24 months of therapy. For patients on alendronate, a similar lower incidence of hip fractures was observed at 18 and 24 months of therapy but not at 6 or 12 months. Results for the hip and nonvertebral fracture outcomes were similar.
In summary, the results of this observational study indicate 1) the real-world effectiveness of risedronate and alendronate through two years of treatment are consistent with results of randomized controlled trials; 2) the reduction of hip and nonvertebral fractures, relative to the referent cohort, occurs earlier after initiation of therapy with risedronate than with alendronate. Given the low adherence to therapy for chronic medications, the consequence of our observation may impact the cost/effectiveness perspective of each bisphosphonate.[1] Silverman et al. Osteoporosis Int. 18:25-34 (2007)

Disclosures: ***P. Delmas****, P&G 2.*
This study received funding from: The Alliance for Better Bone Health (Procter & Gamble Pharmaceuticals and sanofi-aventis).

SA405

See Friday Plenary number F405.

SA406

Homocystinuria Associated Bone Disease: Effect of Long Term Bisphosphonate Treatment. M. D'Amours*, A. Räkel*, L. G. Ste-Marie. Endocrinology, Hôpital Saint-Luc, CHUM, University of Montreal, Montreal, QC, Canada.

Homocystinuria is an autosomal recessive disease classically associated with dislocation of the eye lens, mental retardation, hyperhomocysteinemia and skeleton abnormalities such as deformities, vertebral fractures and osteoporosis. The mainstream of treatment consists of dietary amino acids and vitamin supplementation. There is little data in the literature about the effects of bone active agents such as bisphosphonates in this population. Here we report the cases of 2 brothers diagnosed with homocystinuria in childhood (one at age 5 years and the other at 4 months) who were addressed initially for decreased bone density. They were treated with betain, folic acid, vitamin B12 and calcium. Both had a normal development and growth without fractures or deformities except for a moderate scoliosis in the eldest. The biochemical work-up did not show any abnormality of the phosphocalcic metabolism including PTH and 25(OH)vitaminD. Both presented a slightly elevated level of urinary hydroxyproline suggesting increased bone resorption. Their initial bone mineral density (BMD) showed osteopenia affecting lumbar spine (L2-L4: 0.850 and 0.931 g/cm^2 respectively) and femoral neck (0.965 and 1.055g/cm^2 respectively). Alendronate (ALN) was introduced in 1996 in both patients (then aged 23 and 18 year old).Treatment was continued for the next 10 years without any incident fracture. Sequential lumbar spine BMD was unreliable in the eldest because of progressive scoliosis whereas it increased by 18% in the youngest (1.128 g/cm^2 in 2007). Femoral neck BMD remained stable (2007 values: 0.961 g/cm^2 and 1.047 g/cm^2, respectively). Bone turnover markers, serum osteocalcin (14 and 12ng/ml respectively) and serum C-telopeptide (0,122 and 0,086 ng/ml respectively) were suppressed in these young men. Long term bisphosphonate treatment was associated with the prevention of bone loss and osteoporotic fractures in two patients with homocystinuria. The addition of bisphosphonates to the therapeutic armamentarium for homocystinuria should be considered for fracture prevention.

Disclosures: ***M. D'Amours****, None.*

SA407

See Friday Plenary number F407.

SA408

Patients Desire of Administration Form and Dose Interval in Bisphosphonate Therapy of Osteoporosis. C. Günther, A. Kapner*, C. Spanier*, L. Erich*, V. Schäfer*. Fachklinik Johannesbad, Bad Füssing, Germany.

Introduction: A drug can only work, if you take it in. This platitude is worth on oral bisphosphonate too and the compliance is according to Hadji@al (ECCEO 2005) in daily taken alendronate 27,8% and in weekly taken alendronate 46,5% after 1 year. This cannot satisfy. Before the back ground of a futural available yearly zolendronate intravenous infusion (meanwhile registered as “Aclasta”) we were therefore interested which administration form patients would prefer.
Material and methods: From 19.10.2005 to 05.07.2007 we asked 100 patients with osteoporosis (t-score < -2,5), 91 women (Ø 70,7 years old) and 9 men (Ø 69,5 years old), if they would prefer a daily, a weekly or monthly oral bisphosphonate. After this we began the treatment with the wished oral bisphosphonate under basic therapy with calcium and vitamin D. In addition to the first question we asked the patients if they would prefer a yearly intravenous bisphosphonate infusion if it would be available.
Results: From the 100 patients nobody wanted a daily oral bisphosphonate, 11 patients wanted a weekly drug and 89 patients a monthly. According to the additional question 57 % of the patients would prefer a yearly intravenous zolendronate infusion in comparison to the 43% which would prefer an oral administration.
Discussion: The results show that the majority of patients (89%) prefer the monthly intake of an oral bisphosphonate and only 11% a weekly administration.
If the complete package “Actonel plus Calcium D” - meanwhile available in Germany - can change this relation will be investigated in the next weeks.
Theoretically in the patients who would prefer the yearly zolendronate infusion (57%) could be reached a compliance of 100 % in comparison to the 43% of patients with wished oral administration.
If the “infusion patients” - after registration of “Aclasta” in Germany - switched to infusion indeed will be presented later.
But both application forms - oral and intravenous - will be only effective if a sufficient substitution with vitamin D and calcium is guaranteed. Therefore “Actonel plus Calcium D” in Germany is a good innovation to improve the compliance of patients with osteoporosis.

Disclosures: ***C. Günther****, None.*

SA409

See Friday Plenary number F409.

SA410

Does Alendronate Use Prevent Kyphosis Progression in Older Women? D. M. Kado[1], M. H. Huang*[2], E. Barrett-Connor[3], K. Ensrud[4], A. La Croix*[5], S. R. Cummings[6]. [1]Orthopaedic Surgery, University of California, Los Angeles, CA, USA, [2]University of California, Los Angeles, Los Angeles, CA, USA, [3]University of California, San Diego, La Jolla, CA, USA, [4]University of Minnesota, Minneapolis, MN, USA, [5]University of Washington, Seattle, WA, USA, [6]California Pacific Medical Center, San Francisco, CA, USA.

Hyperkyphosis, or increased thoracic curvature, is commonly observed in older persons. While it is well established that alendronate use decreases the incidence of vertebral fractures and prevents height loss, it is not known whether alendronate use reduces progression of kyphosis. Given that many older women are concerned about the cosmetic deformity known commonly as the dowager’s hump, it is of interest to know whether alendronate actually helps mitigate the progression of age-related kyphosis, especially since a substantial number of the most severely affected women have no evidence of underlying vertebral fractures. Therefore, we compared the effects of alendronate to placebo on the progression of kyphosis in 6,459 women aged 55-81 with low bone mass (femoral neck T-score < 0.68 g/cm^2) using data from the Fracture Intervention Trial (FIT), a randomized blinded trial of alendronate. The kyphosis angle was measured using the Debrunner kyphometer at baseline and closeout in 98% of the participants after an average of 4.2 years.
There were no significant baseline differences between the alendronate and placebo groups in terms of demographic, physical measures, health status, bone mineral density, prevalent vertebral fractures, or kyphosis angle. The mean kyphosis angle was 47.6 degrees (SD = 11.9) and the mean change was an increase of 3.9 (SD = 9.3). For women who did not have baseline vertebral fracture, the average change of kyphosis was 3.9 degrees for both treatment and placebo groups (mean difference = -0.20 degrees, 95% CI: -0.69 - 0.28; p = 0.42). For women who had a baseline vertebral fracture, average changes of kyphosis for the treatment and placebo groups were 4.1 and 4.3 degrees, respectively (mean difference = -0.54, 95% CI: -1.36 - 0.28; p = 0.20). Our data do not demonstrate that kyphosis progression is slowed by alendronate treatment; however, 5% of the participants had changes in kyphosis of greater magnitude (>20 degrees) than would be expected over 4 years, given data from prospective cohort studies. As suggested by findings from prior observational studies, these results support the theory that age-related kyphosis is not primarily caused by underlying spinal osteoporosis, and that its progression may mainly be due to other factors besides vertebral fractures and low bone density.

Disclosures: ***D.M. Kado****, Medtronic 4.*

SA411

See Friday Plenary number F411.

SA412

Bisphosphonate-associated Osteonecrosis of the Jaw cases in South Korea. Y. Chung[1], Y. Kwon*[2], J. Lee*[3], D. Kim[4], S. Lee[5], B. Lee*[6]. [1]Endocrinology and Metabolism, Ajou University Hospital, Suwon, Republic of Korea, [2]Oral and Maxillofacial Surgery, Kyung Hee University Hospital, Seoul, Republic of Korea, [3]Dentistry, Ajou University Hospital, Suwon, Republic of Korea, [4]Nuclear Medicine, Kyung Hee University Hospital, Seoul, Republic of Korea, [5]Biochemistry and Internal Medicine, Eulji University School of Medicine, Daejeon, Republic of Korea, [6]Obstetrics and Gynecology, Inha University Hospital, Incheon, Republic of Korea.

Introduction: Bisphosphonate-associated osteonecrosis of the jaw (ONJ) is a rare but serious side effect of bisphosphonate therapy. Case reports of ONJ had been reported in many Western countries but relatively few from Asian countries. Recently, we had experienced five cases of ONJ in South Korea. Three of them were related with osteoporosis and two of them were associated with malignancy.
Case 1: A 74-year-old woman had been treated with weekly oral alendronate for osteoporosis for 5 years. ONJ of the left mandible precipitated by teeth (1st and 2nd molars) extraction. The patient had multiple systemic risk factors including old age and diabetes mellitus.
Case 2: A 72-year-old woman had been treated with weekly oral alendronate for osteoporosis for 3 years. ONJ of the right mandible and left maxilla area precipitated by teeth extraction. The patient had multiple systemic risk factors including old age, diabetes mellitus, rheumatoid arthritis, and glucocorticoid therapy.
Case 3: A 76-year-old woman had been treated with oral alendronate for osteoporosis for 7 years. ONJ of the right mandible precipitated by teeth (canine and premolar) extraction. The patient had multiple systemic risk factors including old age and diabetes mellitus.
Case 4: A 56-year-old man who had been diagnosed as Waldenstrom's macroglobulinemia has been treated with chemotherapeutic agents. Concomitantly, 45mg of pamidronate was injected monthly for 2 years. There was pain and pus drainage in left mandible 1st molar tooth area after spontaneous foliation. The patient had multiple systemic risk factors including chemotherapy and glucocorticoid for malignancy.
Case 5: A 50-year-old man had been diagnosed as multiple myeloma (non-secretory type). He has been treated with chemotherapeutic agents. Concomitantly, 45mg of pamidronate was injected monthly for 3 years. There was pain and pus drainage in right mandible 1st molar tooth area after extraction. The patient had multiple systemic risk factors including chemotherapy and glucocorticoid for malignancy.
Conclusion: Bisphophonate-associated ONJ both in osteoporosis and malignancy might be an emerging issue in Asian countries as well as Western countries.

Disclosures: ***Y. Chung****, Eli Lilly, GSK 1; MSD, Sanofi-Aventis 1, 2, 3, 4; Novartis 1, 4; Hanlim, Yuyu 1, 2, 3.*

SA413

See Friday Plenary number F413.

SA414

Treatment Discontinuation Due to Gastrointestinal Adverse Events and Decreased Bone Mineral Density in Patients Switched from Branded Alendronate to Generic Alendronate. D. Grima*[1], G. Ioannidis[2], A. Papaioannou[3], J. D. Adachi[3]. [1]Center for Osteoporosis and Arthritis Research and Education, Oakville, ON, Canada, [2]McMaster University, Hamilton, ON, Canada, [3]Medicine, St. Joseph's Healthcare - McMaster University, Hamilton, ON, Canada.

Introduction: Generic alendronate was introduced in Canada in July 2005 and within 2 months the conversion from brand to generic alendronate was almost complete. The generic has a different dissolution profile compared to brand alendronate which may impact gastrointestinal (GI) adverse events (AE).
Methods: A chart review study was conducted of postmenopausal women 50 years of age and older who were on brand alendronate prior to July 2005 from a single clinic that specialized in treating patients with osteoporosis. Data collected included GI AEs, bone mineral density (BMD), osteoporosis therapies used, discontinuation, reason for discontinuation and concomitant use of NSAIDs, PPIs and H2 blockers.
Results: A total of 199 women were identified as starting alendronate prior to the introduction of generic alendronate in July 2005 and having visits after this date. Of these 18 switched therapy before July 2005. Of the remaining 181, 53 patients (29%) discontinued alendronate after July 2005, due to GI AEs (27/181) and declining BMD (26/181).
Discussion: Generic alendronate may not be as well tolerated as brand alendronate and should not be considered equivalent in all individuals. This may have implications for treatment adherence and effectiveness, as well as the preferential reimbursement of generic alendronate.

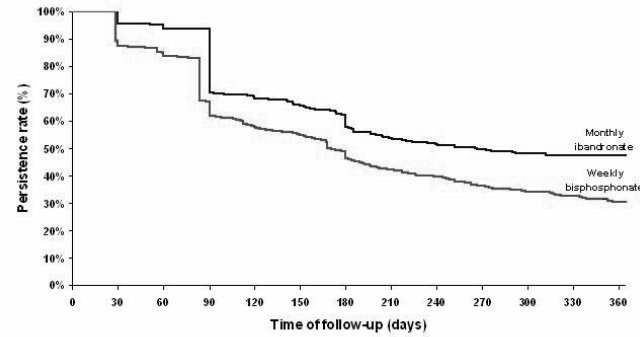
Disclosures: ***D. Grima****, Procter & Gamble Pharmaceuticals 2, 3, 4; sanofi-aventis 2, 3, 4.*
This study received funding from: Procter & Gamble Pharmaceuticals.

SA415

1-Year Analysis of Adherence with Bisphosphonate Treatments in Osteoporotic Patients: Monthly versus Weekly Formulation. C. Roux*[1], F. E. Cotté[2], F. Mercier*[3], A. F. Gaudin[2]. [1]Department of Rheumatology, Paris Descartes University, Paris, France, [2]Economic & Health Outcomes, GlaxoSmithKline France, Marly le Roi, France, [3]StatProcess, Port-Mort, France.

Purpose: To assess medication adherence encompassing both compliance and persistence among women newly receiving monthly ibandronate or weekly bisphosphonates (BPs) in general practice.
Method: Patient data were retrospectively analysed from a computerized database representative of the French GPs prescriptions (1.6 million patients cover). Eligible women were all patients aged at least 45 years with first prescription of BPs (12 months of prior wash-out) from ibandronate availability in France to the end of the follow-up (Jan.2007-Jan.2008). Two cohorts were formed consisting of patients treated with monthly ibandronate and weekly BPs. Compliance was assessed by medication possession ratio (MPR) calculated by dividing the duration of all filled prescriptions by the follow-up period. Persistence was measured by the time to discontinuation therapy (permissible gap in refills were 45 days for monthly and 30 days for weekly regimen due to a two-week difference in dosing window). Treatment survival analyses were completed in both cohorts and analysed by log-rank test. A backwise procedure was used to adjust for confounders.
Results: A total of 2,990 women were prescribed for the first time a bisphosphonate treatment: 1,001 received monthly ibandronate and 1,989 received weekly BPs. On average, women were significantly younger in the first cohort than in the second one (68.8 SD:10.3 and 70.4 SD:10.3 years old respectively; p<.001). The mean MPR was higher with monthly than with weekly dosing (84.5% vs 79.5%; p<.001). Kaplan-Meier curves showed that the proportion of women still persistent at 1-year with monthly ibandronate was 17 percentage points higher than those persisting on weekly BPs (47.5% vs 30.5%; p<.0001). This difference was also significant using similar permissible gaps in both cohorts (p<.0001). After adjustment for age, history of fracture, co-treatments, co-morbidities and DXA scan, monthly users were 37% (hazard ratio = 0.630 SD:0.0613; P<.0001) less likely to discontinue therapy vs weekly users

.Conclusion: These results demonstrate that monthly ibandronate improved adherence (i.e.



compliance and persistence) of osteoporotic patients compared with weekly bisphosphonates.

Disclosures: ***F.E. Cotté****, GlaxoSmithKline 2, 3.*
This study received funding from: GlaxoSmithKline.

SA416

See Friday Plenary number F416.

SA417

Development, Reliability, and Validity of a New Preference Satisfaction Questionnaire (PSQ). D. T. Gold, R. Horne*, C. Hill*, J. Borenstein*, S. Varon*, D. Macarios*. Amgen Inc., Thousand Oaks, CA, USA.

We developed a questionnaire that assesses preference, satisfaction, and bother with a weekly pill versus a 6-monthly, subcutaneous injection for the treatment of postmenopausal osteoporosis.
Thirty-four questions were developed based on literature review and expert input. For nine items, subjects choose one of: the pill, the injection, or neither, with respect to preference, satisfaction, and bother. For 20 items, subjects specify the degree of bother or satisfaction on a 5 point Likert scale for treatment separately (no comparison between treatments). For the remaining five items, subjects select the degree of agreement/disagreement (5 point Likert) with one treatment being favorable over the other on various domains. Two separate, in-depth group interviews were conducted with subjects to evaluate item comprehension, questionnaire length, and identify additional constructs. The PSQ was revised after the first group interviews. Women currently taking or who had previously taken (≤ 3 years) weekly bisphosphonate therapy for osteoporosis were eligible for interviews. Reliability and validity were assessed in a separate study, by incorporating the PSQ into a phase 3 randomized clinical study comparing 60 mg denosumab every 6 month

injection and 70 mg alendronate weekly pill.
Twenty-four subjects participated in initial cognitive interviews. Participants understood the PSQ and did not feel the questions were confusing or contained irrelevant information. 1100 subjects completed the PSQ as part of the clinical study. The construct validity of the PSQ items was investigated through inter-item correlations and correlations between the PSQ items and the EQ-5D. Inter-item correlations supported convergent validity and were for preference items (range 0.62 to 0.97), pill satisfaction items (range 0.23 to 0.90), and injection satisfaction items (range 0.13 to 0.91). Correlations with the EQ-5D were low (range 0.00 to 0.09) supporting divergent validity. The proportion of correlations achieving coefficients of 0.40 or greater among items within the same domain (150/178, 84.3%) is larger than the proportion of coefficients 0.40 or greater among items across domains (122/383, 31.9%), supporting both convergent and divergent validity. Items were retained based on strong inter-item correlations, strong factor loading, and feedback from cognitive interviews. At the scale level, the Cronbach's alpha reliability values for satisfaction, pill-bother and injection-bother were 0.90, 0.85, and 0.62, respectively.
The PSQ was refined and formatted appropriately based on the cognitive interview. Results from a large phase 3 trial show good reliability and validity of the preliminary PSQ.

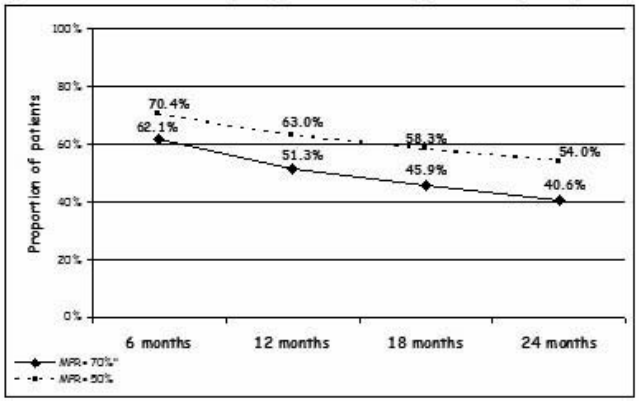
Disclosures: ***D.T. Gold****, Amgen Inc. 1, 2; P&G / Sanofi-Aventis 1, 2, 4; Eli Lilly 1, 2, 4; Merck / Novartis 1, 4.*
This study received funding from: Amgen Inc.

SA418

Patient Persistence with Weekly Bisphosphonates for Two Years after Initiation of Therapy: A Retrospective Cohort Study. D. Gold[1], N. Borisov[2], R. Sheer*[2]. [1]Duke University Medical Center, Durham, NC, USA, [2]Procter & Gamble Pharmaceuticals, Mason, OH, USA.

Therapy persistence is important for a patient to maximize efficacy benefit of medications. The study objective was to assess patient persistence with bisphosphonates during two years of therapy using two integrated administrative medical claims databases (Ingenix Lab/Rx™ and Medstat MarketScan®).
The study included women 50+ years with a new prescription fill for weekly bisphosphonate (risedronate 35mg or alendronate 35/70 mg) between January 1, 2003 and June 30, 2005. To qualify for the study, patients were required to be continuously enrolled for 6 months prior to and 24 months following the index date (the first script date). The 6-month history was used as a wash-out period and to assess baseline clinical characteristics. During follow-up patient's adherence was defined as a medical possession ratio (MPR) of at least 70%. Proportion of patients with MPR ≥ 70% was assessed at 6, 12, 18, and 24 months of therapy. Patients with MPR < 70%, were examined further to assess proportion of patients who reinitiated and remained on the therapy for at least 6 months at any time during follow up. Sensitivity analysis was conducted using different levels of MPR.
We identified 134,032 women with a new prescription for bisphosphonate therapy; mean age was 65 years (SD = 11). Just over half (51%) were persistent with therapy at 12 months, and 41% were still persistent at 24 months after initiation of therapy (Figure 1). Overall, 59% (79,630) of women had MPR < 70% during the follow-up, however, 20% (15,632) of them were persistent with therapy for at least another 6 months, and 8% (6,483) for at least 1 year at some point during follow up. The study demonstrated a decline in patient persistence to approximately 40% over 24 months of bisphosphonate therapy. Although about 60% of women were not persistent with therapy during the follow up, 20% (12% overall) reinitiated and stayed on therapy for at least 6 months. Thus, studies that include efficacy inputs (e.g., cost-effectiveness) should not ignore this cohort of re-starters that attains efficacy for at least another 6 months.

Figure 1 Patients on bisphosphonate therapy over 2-year period



Disclosures: ***D. Gold****, P&G 2.*
This study received funding from: The Alliance for Better Bone Health (Procter & Gamble Pharmaceuticals and sanofi-aventis)

SA419

Hip Structure Analysis for Raloxifene Treatment in Japanese Women with Osteoporosis. J. Takada[1], T. J. Beck[2], T. Miki[3], Y. Imanishi[4], K. Nakatsuka[5], H. Wada*[6], H. Naka*[3], K. Iba[1], T. Yoshizaki*[7], T. Yamashita*[1]. [1]Orthopedic Surgery, Sapporo Medical University, Sapporo, Japan, [2]Radiology, The Johns Hopkins University, Baltimore, MD, USA, [3]Geriatrics, Osaka City University, Osaka, Japan, [4]Metabolism, Endocrinology, and Molecular Medicine, Osaka City University, Osaka, Japan, [5]Inoue Hospital, Suita, Japan, [6]Wada Obstetrics and Gynecology Clinic, Asahikawa, Japan, [7]Kitago Orthopedic Clinic, Sapporo, Japan.

Hip Structure Analysis (HSA) was used to measure proximal femur geometry using conventional DXA scans. This study is the first analysis of HSA data in Japanese osteoporotic women treated with raloxifene (60 mg).
198 Japanese osteoporotic women aged between 47 and 83 years of age were treated with raloxifene for at least 6 months. DXA scans were acquired at baseline and every 6-12 months of follow-up. Geometry and BMD were measured at the narrowest point of the neck (NN), intertrochanteric region (IT), and proximal shaft (PS). Measurements included BMD, cortical thickness, cross-sectional area (CSA), section modulus, and buckling ratio. Section modulus is calculated cross-sectional moment of inertia divided by the maximum distance from the bone edge to the centroid (d_{max}), and represents the index of bending and torsional strength. Buckling ratio is an index of cortical stability under compressive loads, and calculated as the ratio of d_{max} to the estimated mean cortical thickness.
The percent changes of BMD between baseline and year 1 at NN, IT, and PS regions were 0.47 %, 2.73 %, and 1.77 %, respectively. Section moduli were 2.53 %, 4.62 %, and 2.56 %, respectively. Buckling ratios were 0.47 %, -2.36 %, and -1.26 %, respectively. Section modulus has a positive relationship with BMD (r = 0.535 - 0.794, p<0.001), cortical thickness (r = 0.561 - 0.785, p<0.001), and CSA (r = 0.797 - 0.879, p<0.001), but a negative relationship with buckling ratio (r = -0.361 - -0.648, p<0.001) at three measured regions.
In conclusion, raloxifene treatment in Japanese women with osteoporosis increased BMD and improved structure strength in the femur.

Disclosures: ***J. Takada****, None.*

SA420

See Friday Plenary number F420.

SA421

Arzoxifene in Postmenopausal Women with Normal or Low Bone Mass. M. Bolognese[1], J. Krege[2], W. H. Utian*[3], R. Feldman[4], S. Broy[5], J. Alam*[2], D. L. Meats*[2], M. Lakshmanan[2], L. Plouffe*[2], M. Omizo[6]. [1]Bethesda Health Research Center, Bethesda, MD, USA, [2]Lilly Research Laboratories, Indianapolis, IN, USA, [3]North American Menopause Society, Mayfield, OH, USA, [4]Miami Research Associates, Miami, FL, USA, [5]Illinois Bone and Joint Institute, Morton Grove, IL, USA, [6]Oregon Osteoporosis Center, Portland, OR, USA.

Arzoxifene is a benzothiophene estrogen agonist/antagonist that is more potent and bioavailable than raloxifene (Palkowitz et al. 1997). The study was a 24-month, Phase 3, double-blind, multicenter trial of postmenopausal women with femoral neck or lumbar spine bone mineral density (BMD) T-score between -2.5 and 0 randomized to arzoxifene 20 mg/day (N=172) or to placebo (N=159). Elemental calcium 500 mg/day was provided. Primary endpoints were lumbar spine and total hip BMD change and endometrial safety. At baseline, subjects were well matched (Caucasian 83%, mean age 55 years, mean body mass index 28 kg/m2, mean lumbar spine BMD 0.95 g/cm^2, total hip BMD 0.89 g/cm^2, median CTX 0.58 ng/L, and median PINP 52 mcg/L). At 6 months and at subsequent assessments, BMD increases at all skeletal sites were significant (p<0.05) in the arzoxifene vs placebo group. At 24 months, BMD increases in the arzoxifene vs placebo group were lumbar spine 3.2%, total hip 2.3%, femoral neck 2.1%, and trochanter 3.0% (p<0.001 for all comparisons). In the arzoxifene vs placebo group, Lumbar spine BMD (2.9%, p<0.001) and total hip BMD (2.2%, p<0.001) increased from baseline to last-observation-carried-forward endpoint. No significant subgroup treatment effect at any skeletal site was observed (years postmenopausal [>2 and <5 vs ≥5 years], age [<55 vs ≥55 years], and ethnicity [Caucasian vs non-Caucasian]; all p>0.1). At 3 months and at subsequent assessments, CTX and PINP displayed a significant decrease in the arzoxifene vs placebo group (p<0.001), and at 24 months, CTX was decreased by 30% (p<0.001) and PINP was decreased by 31% (p<0.001) in the arzoxifene vs placebo group. There were no significant between-group differences in the incidence of endometrial hyperplasia or cancer as assessed by serial endometrial biopsy (placebo 2, arzoxifene 0) or in endometrial thickness assessed by transvaginal ultrasound. Adverse event monitoring showed no significant increase in adverse events in the arzoxifene group except for the MedDRA term "vulvovaginal mycotic infection" (placebo 0%, arzoxifene 4%, p=0.02). New or worsening hot flashes were not significantly different between the groups (placebo 11%, arzoxifene 12%, p=0.87). There were no deaths or venous thromboembolic events. In postmenopausal women with normal to low bone mass, arzoxifene 20 mg/day demonstrated significant skeletal efficacy and was well tolerated.

Disclosures: ***M. Bolognese****, Eli Lilly and Company 1.*
This study received funding from: Lilly Research Laboratories.

SA422

Excess Medical Cost after a Fragility Fracture during 3-year Follow-up: Medicare Perspective. D. Brixner*[1], N. Borisov*[2], C. Purple[2]. [1]University of Utah, Utah, UT, USA, [2]Procter & Gamble Pharmceuticals, Mason, OH, USA.

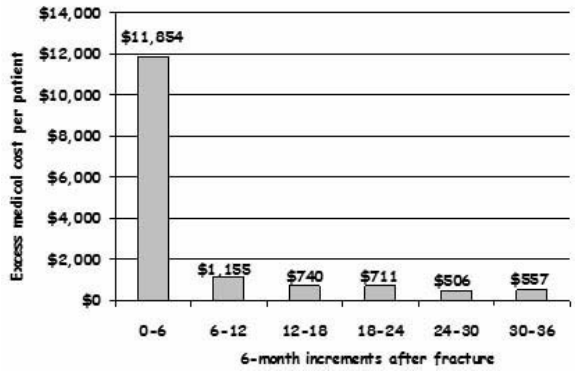
The medical costs of a fragility fracture are high in the year following the fracture (Ohsfeldt, 2006). This study estimated long-term fracture-related excess medical cost incurred during 3 years following fragility fracture compared to the cost incurred before the fracture.

Using medical claims database (Medstat MarketScan®), we conducted a retrospective cohort study among women 65+ years with a new Medicare claim for a closed non-traumatic fracture (index) at any of 7 anatomical sites: hip, leg, humerus, clavicle, pelvis, wrist, or spine between July 1, 2000 and June 30, 2005. Women with a claim for malignant neoplasm, radiation oncology or chemotherapy were excluded. The cohort was followed in 6-month increments over 3-year follow up period. To estimate fracture excess cost each post-fracture time increment was compared to the 6-month before the fracture. The excess cost was examined by place of service, fracture site, and subsequent fractures and reported in 2007 US dollars.

Out of 1,665,837 eligible women 65+ in the database during the study period, we identified 31,758 (3%) women with a Medicare claim for the specified fragility fractures. The total fracture excess medical cost was $15,522 per patient during 3-year follow-up. Most (76%) of the excess cost was incurred during the first 6 months after the fracture (Figure 1) with costs in inpatient hospital and long term care contributing 55% and 28%, respectively. The excess prescription cost ($590/patient) contributed 4% to the total excess cost. Nonvertebral fractures accounted for 85% of all fractures (vertebral and nonvertebral) and for 87% ($13,469/patient) of the total excess cost. Wrist, hip, humerus, and clavicle fractures incurred excess medical costs continuously over each 6 month period of the follow-up; whereas leg, spine and pelvic fractures did not have excess costs beyond 24 months. About 14% (4,419) of women experienced a subsequent fracture during the follow-up with a total $28,021 in excess medical cost per patient during the entire 3-year period after the index fracture.

Although most of the excess medical cost attributable to fragility fractures occurred in the first 6 months, the cost did not return to the baseline over the 3-year follow-up. This demonstrates the long-term nature of the fragility fracture costs.

Figure 1. Fracture-related excess medical cost



Disclosures: ***D. Brixner****, P&G 2.*

This study received funding from: The Alliance for Better Bone Health (Procter & Gamble Pharmaceuticals and sanofi-aventis).

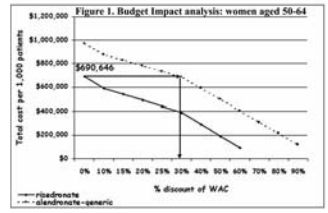
SA423

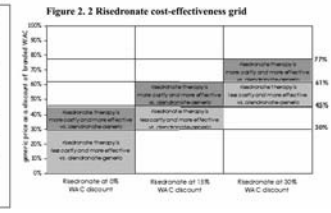
Cost-Effectiveness of Risedronate vs. Generic Alendronate: 1-year Analysis among Women 50-64 Years Old. D. Grima*[1], N. Borisov[2]. [1]Cornerstone Research Group Inc, Burlington, ON, USA, [2]Procter & Gamble Pharmaceuticals, Mason, OH, USA.

Generic alendronate to treat osteoporosis was introduced to the US market in February, 2008, at price discounts of 10% to 44% of branded alendronate. A lower price of generic may introduce some savings to a managed care (MC) budget, however, to measure the true cost, therapy's copayments, rebate discounts, and efficacy should be considered. We used a model of postmenopausal osteoporosis to compare the budget impact of risedronate to generic alendronate at a range of generic wholesale acquisition cost (WAC).

The analysis included women 50-64 years, with T-scores <-2.5, under a 1-year time horizon from MC perspective. Fracture rates and costs were derived from US sources. Vertebral and nonvertebral risk reductions were 59% and 47%, respectively, for alendronate and 65% and 74%, respectively, for risedronate. The budget impact analysis included (1) *Net Drug Cost* (NDC), WAC adjusted for copayment and rebate discount, and, (2) *Fracture-related Cost*. The discounts on both products were varied independently from 0% to 90%. Annual WAC (before adjustment) and copayment were $1,001.39 and $390 for risedronate and $947.50 and $130 for branded alendronate, respectively.

The results found risedronate's NDC below generic's NDC at every level of WAC discount due to a higher annual copayment. The difference in copayment and fracture efficacy, resulted in price equivalence of risedronate with no discount to generic alendronate at 30% discount (Figure 1). The efficacy advantage of risedronate translated into cost savings or cost-effectiveness in a wide number of rebate discount scenarios (Figure 2)





.Given current generic alendronate price discount (10%, 19%, 44% of the branded WAC by Watson, Barr, and Teva, respectively), risedronate is more effective and cost-saving without any discounts compared to the Watson and Barr generics and more effective and cost-effective compared to the Teva generic. In case of generics reducing their prices further (up to 61% of branded WAC), risedronate would remain cost-effective at 15% rebate.

Disclosures: ***D. Grima****, P&G 5.*

This study received funding from: The Alliance for Better Bone Health (Procter & Gamble Pharmaceuticals and sanofi-aventis).

SA424

Cost-Effectiveness of Risedronate versus Ibandronate at One Year: The Case of the United Kingdom. D. Farquhar*, M. Pasquale*. Procter & Gamble, Mason, OH, USA.

Background/Objectives: A recent observational cohort study directly comparing risedronate to ibandronate showed that women initiating treatment with risedronate had a lower incidence of hip fractures than women initiating treatment with ibandronate (Ringe, 2008). The incidence of hip fracture during the first year of therapy was 0.37% for risedronate and 0.81% for ibandronate (adjusted relative risk for risedronate vs. ibandronate: 0.51 (95% CI 0.30 - 0.87)). Despite limitations in interpretation of observational cohort studies due to the non-randomized nature of the study design, the objective of this analysis was to determine the cost-effectiveness of risedronate compared to ibandronate using these effectiveness data for the case of patients with confirmed osteoporosis in the United Kingdom (UK).

Methods: A validated Markov model of osteoporosis (Tosteson, 2001) was used to estimate the impact of therapy on hip fractures and costs. The model simulated a cohort of 1,000 women aged 65+, with BMD≤-2.5 and a previous fracture, treated with once-a-week dosing of risedronate or ibandronate over one year. UK data included annual drug cost (risedronate £264.60/year; ibandronate £257.40/year) and one-year fracture costs.

Results: In a cohort of 1,000 women treated with risedronate versus ibandronate, the model predicted 11 fewer fractures and a cost savings in total medical costs (drug plus fracture treatment) of £148,160. Risedronate dominates ibandronate in cost-effectiveness. Cost savings by age are specified in Table 1.

Extrapolating this result to the population of women aged 65+ in the UK initiating osteoporosis therapy suggests that risedronate would prevent an additional 1,340 hip fractures in the first 12 months of therapy and a cost savings of over £18 million, compared to ibandronate.

Table 1. Total Medical Cost Savings with Risedronate vs. Ibandronate (Cohort of 1,000)

Age Group	Number of Hip Fractures	Difference in Hip Fractures	Total Medical Cost(Drug + Fracture Treatment)	Cost Savings with Risedronate vs. Ibandronate
65-74	Ris: 4.47 Iban: 7.21	2.74	Ris: £177,848 Iban: 205,569	£27,721
75-84	Ris: 7.27 Iban: 11.73	4.46	Ris: £150,759 Iban: £218,551	£52,647
85+	Ris: 6.44 Iban: 10.39	3.95	Ris: £150,759 Iban: £218,551	£67,792
Total	Ris: 18.18 Iban: 29.33	11.15	Ris: £513,461 Iban: £661,621	£148,160

Disclosures: ***D. Farquhar****, P&G 2.*

This study received funding from: The Alliance for Better Bone Health (Procter & Gamble Pharmaceuticals and sanofi-aventis).

SA425

Cost-Effectiveness of Risedronate versus Ibandronate at One Year: The Case of Germany. J. Brecht*[1], M. Pasquale[2], W. Moehrke[3], H. Kruse*[4]. [1]InForMed – Outcomes Research and Health Economics, Ingolstadt, Germany, [2]Procter & Gamble Pharmaceuticals, Mason, OH, USA, [3]Procter & Gamble Pharmaceuticals, Schwalbach, Germany, [4]Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany.

Background/Objectives: Recently an observational cohort study directly comparing risedronate to ibandronate showed that women initiating treatment with risedronate had a lower incidence of hip fractures than women initiating treatment with ibandronate (Ringe, 2008). The incidence of hip fracture during the first year of therapy was 0.37% for risedronate and 0.81% for ibandronate. Statistically, relative to ibandronate patients, the adjusted relative rate of hip fracture for risedronate patients was 0.51 (95% CI 0.30 - 0.87). The objective of this analysis was to determine the cost-effectiveness of risedronate compared to ibandronate using these effectiveness data for the case of osteoporotic women in Germany.

Methods: A validated Markov model of osteoporosis (Tosteson, 2001) was used to estimate the impact of therapy on hip fractures and costs. The model simulated a cohort of 1,000 women aged 65+, with BMD≤-2.5 and a previous fracture, treated with once-a-week dosing of risedronate or ibandronate over one year. German data included annual drug cost (risedronate €468.32/year; ibandronate €571.20/year) and one-year hip fracture costs €23,895 (Brecht, 2003).
Results: In a cohort of 1,000 women aged 65+ treated with risedronate versus ibandronate, the model predicted 6 fewer hip fractures and a cost savings in total medical costs (drug plus fracture treatment) of €282,520, for patients treated with risedronate versus ibandronate (Table 1). Risedronate dominates ibandronate in cost-effectiveness. Extrapolating this result to the population of women aged 65+ in Germany initiating bisphosphonate therapy suggests that risedronate would prevent an additional 1,899 hip fractures in the first 12 months of therapy at a cost savings of over €89 million, compared to ibandronate.
Conclusions: Based on "real world" observational data, this analysis of post-menopausal women in Germany aged 65+ suggests risedronate provides more fractures averted at a lower total medical cost, resulting in substantial cost savings compared to treatment with ibandronate.

Table 1. Fracture and Medical Cost Savings with Risedronate vs. Ibandronate (Cohort of 1,000)

Age Group	Fractures Averted with Risedronate vs. Ibandronate	Total Medical Cost (Drug + Fracture Treatment Cost)	Cost Savings with Risedronate vs. Ibandronate
65-69	3	Ris. €593,24 7Iban. €774,583	€181,336
70-74	4	Ris. €628,297Iban. €831,512	€203,215
75-79	9	Ris. €808,609Iban. €1,123,067	€314,215
80+	10	Ris. €842,505Iban. €1,179,303	€336,798

Disclosures: ***J. Brecht****, P&G 2.*
This study received funding from: The Alliance for Better Bone Health (Procter & Gamble Pharmaceuticals and sanofi-aventis).

SA426

See Friday Plenary number F426.

SA427

Modeled Cost-Effectiveness of Risedronate versus Ibandronate: The Case of Italy. S. Maggi*[1], M. Pasquale[2], O. Bouin*[3]. [1]CNR Aging Branch,University of Padua, Padua, Italy, [2]Procter & Gamble Pharmaceuticals, Mason, OH, USA, [3]sanofi-aventis, Paris, France.

OBJECTIVE: Recently an observational cohort study directly comparing risedronate to ibandronate showed that women initiating treatment with risedronate had a lower incidence of hip fractures than women initiating treatment with ibandronate (Ringe, 2008). The incidence of hip fracture during the first year of therapy was 0.37% for risedronate and 0.81% for ibandronate (adjusted relative risk 0.51, CI: 0.30-0.87). The objective of this analysis was to determine the cost-effectiveness of risedronate compared to ibandronate using these effectiveness data for the case of patients with confirmed osteoporosis in Italy.
MATERIAL AND METHODS: A validated Markov model of osteoporosis (Tosteson, 2001) was used to estimate the impact of therapy on hip fractures and costs from the Italian National Health System (NHS) perspective. The analysis included 75 year-old women treated with risedronate or ibandronate for 1 year. The model further simulated downstream costs for an additional 5 years. Country-specific data included general population hip fracture incidence rates and mortality, hip fracture costs (€11,571 in the first year, and €1,320 in subsequent years per fracture, Piscitelli, 2006), and annual drug costs (risedronate €36.34 per box and ibandronate €43.7 per box). Costs and outcomes were discounted at 3%. A differential relative risk reduction of 49% was applied to risedronate vs. ibandronate (Ringe, 2008).
RESULTS: In a cohort of 1,000 post-menopausal osteoporotic women with 1 year of treatment the model predicted 8 fewer hip fractures, 7 more QALYs, and a cost savings of €187,407 for risedronate compared to ibandronate. If extrapolated to a population of Italian osteoporotic women aged 70-79, this analysis suggests that 648 additional hip fractures can be avoided and over €14.6 million saved by treating patients with risedronate rather than ibandronate. Sensitivity analysis on the cost of hip fracture confirmed dominance of risedronate versus ibandronate in this patient population.
CONCLUSIONS: Based on Italian epidemiological data and "real world" observational effectiveness data, this analysis of post-menopausal women in Italy suggests risedronate provides more fractures averted at a lower total medical cost, resulting in substantial cost savings compared to treatment with ibandronate.

Disclosures: ***S. Maggi****, P&G 2.*
This study received funding from: The Alliance for Better Bone Health (Procter & Gamble Pharmaceuticals and sanofi-aventis).

SA428

Physician Practices in Bone Density Testing among Medicare Patients. J. M. Neuner[1], X. Zhang*[2], R. Sparapani*[2], P. W. Laud*[1], A. B. Nattinger*[1]. [1]Medicine, Medical College of Wisconsin, Milwaukee, WI, USA, [2]Biostatistics, Medical College of Wisconsin, Milwaukee, WI, USA.

Purpose Medicare has recently added bone density testing of women 65 and older to its pay-for-performance program, but little is known about current testing. We sought to determine the rate of testing among physician practice panels and the contribution of both physician and physicians' practice variables to BMD testing.
Methods: Women aged ≥ 65 enrolled in Medicare between 1999-2003 in CA, FL, IL, or NY were identified using Medicare administrative data. Physicians from these states were identified as "plurality of care" physicians if they saw subjects at least twice over a two-year period, and more than any other general internal medicine, obstetrician/gynecologist (GYN), family medicine, or general practice physician. Logistic regression models of factors associated with subject receipt of ≥1 bone density test (ultrasound, central or peripheral DXA) in 2002-2003 was developed with the physician and his/her patient panel as the units of analysis.
Results: The 27,882 physicians (51% internal medicine, 32% family medicine, 10% GYN, 6% general practice) were plurality of care physicians for a median of 52 subjects. The median proportion of subjects in a physician panel with bone density tests in 2002-2003 was 27% (interquartile range 16-45%) In a regression model including only physician factors and state (table), the strongest individual predictor of bone density testing was GYN specialty. When patient panel variables were added to the model, specialty effects were reduced for gynecologists (OR 2.59 95% CI 2.53, 2.64) but increased for other specialties. The effects of other physician characteristics on testing were all slightly reduced in the larger model. Higher patient panel age, income, visit number, and percentage of black or Hispanic patients, and lower percentage of Medicaid recipients were all associated with more testing.
Conclusions Physician characteristics are strongly associated with bone density testing, but these associations are somewhat attenuated by physician's patient panel characteristics. Given previously described age and racial/ethnic testing disparities and limited data regarding patient preferences, pay-for-performance initiatives may need to emphasize improvements in addition to performance thresholds.

Association of Physician Characteristics with Bone Density Testing

Physician Characteristics		Odds Ratio	95% CI
Specialty	GYN	3.38	{3.31, 3.44}
	Internal Med	1.49	{1.47, 1.52}
	Family Med	1.23	{1.21, 1.25}
	General Practice	reference	
Female Sex		1.28	{1.27, 1.29}
Panel size >median		1.30	{1.29, 1.31}
Years in Practice>median		0.84	{0.83, 0.84}

Disclosures: ***J.M. Neuner****, None.*
This study received funding from: National Institute on Aging.

SA429

Cost-Effectiveness of Ibandronate Therapy for Women with Postmenopausal Osteoporosis with Respect to Nonvertebral Fracture Efficacy. J. A. Sunyecz*[1], C. Silberman[2], S. Poston*[3], S. Earnshaw*[4]. [1]Laurel Highlands Ob/Gyn, Hopwood, PA, USA, [2]Roche, Nutley, NJ, USA, [3]GlaxoSmithKline, Research Triangle Park, NC, USA, [4]RTI, Research Triangle Park, NC, USA.

Bisphosphonate trials in osteoporosis are typically powered to examine change in bone mineral density and/or vertebral fracture incidence. Efficacy against nonvertebral fractures, which are less frequent, is more difficult to demonstrate. A pooled analysis of ibandronate clinical trials has been performed in which the impact of ibandronate therapy on nonvertebral fractures was estimated. In this analysis, we developed a Markov model to examine cost-effectiveness of ibandronate in postmenopausal women with established osteoporosis (vertebral fracture and bone mineral density T-score ≤-2.5; mean age 78 years) while considering nonvertebral fracture efficacy. The model, based on 5 years of therapy and a lifetime time horizon, compared cost-effectiveness of ibandronate and other bisphosphonates versus no bisphosphonate therapy. Bisphosphonate efficacy was obtained from published meta-analyses. Fracture risks, mortality, resource use, costs, and utilities were obtained from the literature. Persistence rates at 3, 6, 9, and 12 months for patients on monthly bisphosphonate therapy were derived retrospectively from managed care claims. For modeling purposes, other bisphosphonate regimens were assumed to have similar persistence rates to monthly ibandronate. Persistence was extrapolated to a maximum of 5 years. Following discontinuation, treatment benefit declined linearly and proportionally to the duration of active treatment. Yearly drug cost was referenced to wholesale acquisition cost and assumed to be the same for all bisphosphonates. Costs and outcomes were discounted 3% annually. All costs were reported in 2007 US$. Bisphosphonate therapy conferred improvements in quality-adjusted life years (QALYs) versus no bisphosphonate therapy; ibandronate patients had greater QALY increases than patients on other bisphosphonates (0.0233 vs 0.0197). Fracture care costs were less per patient with ibandronate ($7,652 vs. $8,308) or other bisphosphonates ($7,816 vs. $8,308) than without any bisphosphonate therapy. Drug costs per patient were similar between ibandronate and other bisphosphonates--$855 per year. Incremental cost/QALY gained (vs. no therapy) was lower with ibandronate ($8,512) than with other bisphosphonate therapy ($18,431). Ibandronate treatment thus resulted in cost-savings compared with other bisphosphonates. Our Markov model predicts that reduced incidence of key nonvertebral fractures with ibandronate may reduce fracture care costs for improved cost-effectiveness in comparison to other bisphosphonates.

Disclosures: ***J.A. Sunyecz****, GlaxoSmithKline, Roche 1.*
This study received funding from: Roche and GlaxoSmithKline.

SA430

Falls Predict Higher Medical Costs among Osteoporosis Patients in Community Setting. A. I. Wertheimer*[1], T. Fan*[2], S. S. Sen*[2], S. Rajagopalan*[3]. [1]School of Pharmacy, Temple University, Philadelphia, PA, USA, [2]Global Outcomes Research, Merck & Co., Inc, Whitehouse Station, NJ, USA, [3]Med Data Analytics, Inc., New York, NY, USA.

OBJECTIVE: To estimate the direct medical costs of falls in a population of community-dwelling patients with osteoporosis.
METHODS: Data from a sample of 462 community-dwelling patients with self-reported diagnosis of osteoporosis from the 2004 Medical Expenditure Panel Survey were included to estimate their fall-associated medical expenses. The results were projected to the entire population of civilian, non-institutionalized elderly in the United States.
RESULTS: In 2004, among patients with osteoporosis, 13.6% of the non-institutionalized elderly osteoporotic patients in the United States reported medical conditions related to falls. The estimated total direct medical cost of these conditions was $1.00 billion - 1.43 billion in 2004 dollars. The mean cost per person who had fallen was $2,974 in 2004 dollars. Inpatient hospitalizations accounted for 45.6% of total costs, followed by office-based medical visits and home health care, each accounting for about 10% of total direct medical costs, and hospital outpatient visits for 7.6%. About 48.1% of fall-related costs were reimbursed by Medicare. In general linear regression models, a history of fall was a significant predictor of medical expenditures for osteoporosis patients, after adjusting for age, gender, race, education level and geographic region (t=-3.13, P=0.0019).
CONCLUSION: Fall-related medical conditions affect a substantial number of community-dwelling patients with osteoporosis and result in an enormous economic burden in the United States. Actual burden of falls may be significantly higher when nonmedical and indirect costs were included. The results of this study highlighted the importance of research about strategies to prevent falls among patients with osteoporosis.

Disclosures: ***T. Fan****, Merck & Co., Inc 5.*

SA431

Effects of Acanthopanax Senticosus Extract on Bone Metabolism In Korean Postmenopausal Women. H. Chung[1], E. Kim[2], S. Yoon*[3], I. Jeong*[1], K. Ahn*[1], M. Kwon*[1], s. Chon*[1], S. Oh*[1], J. Woo*[1], S. Kim*[1], J. Kim*[1], Y. Kim*[1]. [1]Medicine, Kyung Hee University, Seoul, Republic of Korea, [2]Medicine, Fatima Hospital, Daegu, Republic of Korea, [3]Food and Nutrition, Yonsei University, Seoul, Republic of Korea.

Acanthopanax senticosus is a traditional herbal medicine for reducing inflammation, enhancing liver function and musculosketal system. In preliminary study, Acanthopanax seticosus extract has shown increased osteoblastic cell proliferation, differentiation and bone nodule formation. The purpose of this study was to determine the safety and effect of Acanthopanax senticosus extract as an alternative medicine on bone metabolism in Koean postmenopausal women. The subjects were 81 healthy female with postmenopausal osteopenia and osteoporosis. The subjects were randomly assigned into treatment and control group. Treatment group received Acanthopanax senticosus extract (3g/d) and all subjects were received calcium and vitamin D for 6 months. DXA of spine and hip were obtained at baseline and at 6 months. Level of serum C-telopeptide and osteocalcin were also measured as bone markers. Safety was assessed by recording of all adverse events and regular monitoring of blood biochemical tests. In the treatment group, bone mineral density did not increased at spine and femur compared with the control group. At 6 months, however, levels of serum osteocalcin significantly increased in the treatment group. There are no significant differences of adverse events and biochemical blood tests between the groups. This study suggest that Acanthopanax senticosus extract is safe as an alternative herbal medicine and may have a role in bone metabolism. However, further study for longer period to see BMD changes should be performed.

Disclosures: ***H. Chung****, None.*
This study received funding from: Korea Health Industry Development Institute.

SA432

The Effect of a One Year Exercise Program on Markers of Bone Metabolism after Hip Fracture. J. Yu-Yahiro[1], E. Streeten[2], M. Hochberg[2], D. Orwig*[2], J. Magaziner[2]. [1]Department of Orthopaedics, Union Memorial Hospital, Baltimore, MD, USA, [2]Epidemiology and Preventive Medicine, University of Maryland School of Medicine, Baltimore, MD, USA.

A study has been conducted to evaluate the impact of home-based exercise over one year in minimizing losses in bone and muscle. This project compared markers of bone metabolism in hip fracture patients randomized exercise or usual care.
Community dwelling women, with fresh femur fractures were randomized to exercise and usual care groups (N=75 each). Exercisers began the intervention at the end of the usual post-acute physical therapy (mean 72 ± 4.5 days). Exercise was five, 45 minute sessions per week, 3 aerobic and 2 strength training, including a maximum of 56 trainer-supervised sessions decreasing in frequency from the beginning to the end of program.
Serum was collected at baseline (within 15 days of fracture), and 60, 180, and 360 days post fracture. Samples were processed and stored at -70C and analyzed for bone specific alkaline phosphastase (BAP) by immunoradiometric assay, and C-terminal propeptide of type 1 collage (CICP) and carboxyterminal cross-linked telopeptide of type 1 collagen (CTx) by ELISA. The intention-to-treat principle was followed and generalized estimating equations were used to perform repeated measures analyses with time and intervention as fixed effects.
Of exercisers, 81% were actively followed by a trainer and 18% refused participation after being enrollment. Participants received an average of 44 (78%) of the prescribed visits by the exercise trainer.
Although markers of osteoblastic activity, BAP and CICP appeared to decline over time in both exercisers and controls, there were no significant differences in levels of either marker over time (Table 1). Likewise, there were no differences between the two groups at 2, 6, or 12 months. CTx levels were similar in controls and exercisers at all points with no differences found overtime in either group.
In conclusion, compared to a group receiving usual care post fracture, participation in a year long program of exercise after hip fracture did not cause changes in osteoblastic or osteoclastic activity as evidenced by measurement of three markers of bone metabolism.
Table 1: Serum Markers of Bone Metabolism and Exercise Intervention

Group	2 months mean (SE) n	6 months mean (SE) n	12 months mean (SE) n	Between Group Differences
Control BAP	18.42 (1.65) 56	14.65 (1.42) 57	11.93 (1.03) 53	
Exercise BAP	17.84 (2.11) 51	13.35 (0.93) 60	10.97 (0.87) 55	p=0.91
Control CICP	108.9 (8.3) 56	98.1 (6.8) 57	77.5 (5.7) 53	
Exercise CICP	116.9 (11.9) 51	89.9 (5.8) 60	81.6 (5.4) 55	p=0.16
Control CTx	0.74 (0.05) 56	0.64 (0.05) 57	0.52 (0.04) 53	
Exercise CTx	0.74 (0.05) 51	0.55 (0.03) 60	0.54 (0.04) 55	p=0.103

All time specific differences n.s. at the .05 level.
Note: all post-randomization values (2 months to 12 months) were adjusted for baseline (pre-randomization) values.

Disclosures: ***J. Yu-Yahiro****, None.*
This study received funding from: National Institute on Aging.

SA433

See Friday Plenary number F433.

SA434

Male Perspectives on Osteoporosis Suggest that Continuous Followup and Tailored Groups for Patient Education Are Important Issues - Focus Group Interviews. D. S. Nielsen[1], K. Brixen[2], L. Huniche*[3]. [1]Endocrinology, Odense University Hospital, Odense, Denmark, [2]Institute of Clicical Research, University of Southern Denmark, Odense, Denmark, [3]Health, Man & Society, Institute of Public Health, University of Southern Denmark, Odense, Denmark.

Several studies have confirmed that osteoporotic fractures in men are an increasing public health problem. Male osteoporosis, however, is underdiagnosed and undertreated. The purpose of this study was to gain insight into how men experience being diagnosed with osteoporosis and how they handle osteoporosis in their everyday lives. In particular focus was directed on information, patient education, and patients' acceptance of treatment.
Methods, Data were collected from 4 focus groups with a total of 16 men aged 52-82 years diagnosed with osteoporosis. Data analysis aimed to elicit knowledge derived from the men's everyday lives and experiences. A thematic analysis approach was used. The analysis was discussed with other researchers, in order to develop analytical categories and interpretations.
Results. The interviews illustrated that information, patient education, and acceptance of treatment are of varying importance and utilised in different ways in the everyday lives of men with osteoporosis.

Problem	Impact on everyday life
Difficult to tell friends, family and colleagues about having osteoporosis - osteoporosis is considered a female's disease	Barrier to implement lifestyle changes and abstain from heavy lifting and strenuous jobs - It is important for most men to appear as strong and masculine.
In health promotion scarring campaigns are often used.	Professionals should have a concerned and empathetic approach to patients - Important to patient's motivation, and to how patients handle osteoporosis in the every day life.
The health care system is offering mainly individual consultations.	Most of the men suggested group-based patient education programs. Some preferred being together with women due to women's way of being open about feelings. Others preferred men-only groups to enhance the sharing of experiences.
At times, participants felt it difficult to take the medication.	Motivation fades out with time. A continuous follow up (e.g. once a year) at the doctor or osteoporosis specialist nurse was considered as very important.

Conclusion. The study underscores the importance of working with the everyday context and perspective of the patient in the health care system. A better understanding of how osteoporosis affects men may help to develop specific preventive strategies as well as enhancing patients' acceptance of treatment.

Disclosures: ***D.S. Nielsen****, None.*

SA435

See Friday Plenary number F435.

SA436

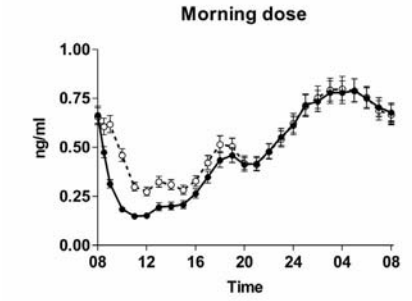
Exploring the Effect of Food Intake On Bone Resorption for Optimal Drug Delivery and Efficacy in Osteoporosis with Oral Calcitonin. M. A. Karsdal, I. Byrjalsen, B. J. Riis*, C. Christiansen. Nordic Bioscience, Herlev, Denmark.

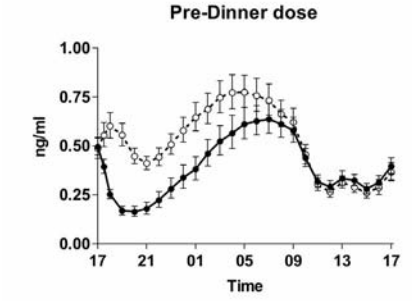
Bone resorption displays marked diurnal variation. Completely reversible inhibition of bone resorption may result in best possible efficacy when bone resorption peaks, i.e. during the night. The aim of the study was to assess the pharmacokinetic (PK) and pharmacodynamic (PD) profiles of 0.8 mg of oral salmon calcitonin (sCT) and the effect of timing of drug intake in healthy postmenopausal women.

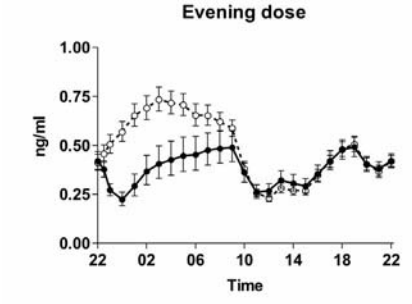
The study was a randomized, double-blind, double-dummy, placebo-controlled, crossover phase I study to assess the pharmacokinetic (PK) and pharmacodynamic (PD) profiles of 0.8 mg of oral sCT in healthy postmenopausal women. 81 subjects were included. Morning dose was given at 8:00 (*n*=42), a pre-dinner dose given at 17:00 (*n*=20) and evening dose given at 22:00 (*n*=19). Blood sampling occurred before drug intake, and at 5, 10, 15, 30, 45 minutes, 1, 1½, 2, 2½, 3 hours, and every hour until 24 hours after dosing. The absorption of calcitonin was assessed by measurement of plasma sCT concentrations, and bone resorption by the biochemical marker serum CTX-1.

Morning and pre-dinner dosing led to comparable concentrations of sCT (45 pg/ml), whereas there was a tendency towards lower C_{max} for the evening dosing having a mean of 24 pg/ml. Overall, dosing with oral sCT resulted in significant suppression of serum CTX irrespective of dosing time, although with different pharmacodymamic parameters (Figure 1). The morning dose of oral sCT resulted in a placebo-corrected $AUC_{0\text{-}24hrs}$ of serum CTX of -232 (% x hrs) corresponding to a permanent overall suppression of bone resorption by 10%. The pre-dinner dose was twice as effective in suppression of serum CTX giving a mean $AUC_{0\text{-}24hrs}$ of -603 (% x hours) equal to an overall suppression of 25%. The $AUC_{0\text{-}24hrs}$ of the evening dose was at the same level of magnitude at -538 (% x hrs) although there seemed to be a higher inter-individual variation in the response associated with this dosing time.

Conclusion: The study suggests that orally administered 0.8 mg of salmon calcitonin was effective in suppression of serum CTX over 24h irrespective of time of dosing. The pre-dinner dosing at 17:00 resulted in optimum efficacy response corresponding to an overall suppression of bone resorption by 25%. In the optimization of efficacy of completely reversible anti-resorptive drugs, the physiological timing of drug dosage is important to optimise drug efficacy.







Disclosures: ***M.A. Karsdal****, None.*

SA437

Osteoporosis in the Old Old: Review of the Evidence. C. A. Inderjeeth, A. Foo*, M. Lai*. Rehabilitation and Aged Care, Sir Charles Gairdner Hospital, Nedlands, Australia.

Women ≥ 80 years of age comprise approximately 8% of the post-menopausal population but contribute > 30% of all fragility fractures and 60% of hip fractures because of the high prevalence of osteoporosis and high incidence of falls in this age group. However there is limited data in this age group for treatment.

Objectives of this review: To review the published literature on the clinical efficacy and safety of specific anti osteoporosis treatments in the reduction in fracture risk in females in the old old (greater than 80 years of age). The following major endpoints were used in this review:

1. Vertebral fracture reduction at 1 year and 3 years
2. Non-vertebral fracture reduction at 1 year and 3 years
3. Hip fracture reduction at 1 year and 3 years.
4. Safety data with these agents in the old old.

Studies had to be randomised placebo or active comparator control trials of at least 1-year duration including post-menopausal females. Pooled analysis and published sub-group analysis, specifying the sub-group 80 years or older were also included.

Results: The only publications that specifically reported data in patients from 80 - 100 years old were pooled analysis of the SOTI and the TROPOS study by Seeman et al and the pooled analysis of VERT MN, VERT NA and HIP study by Boonen *et al*. These results are summarised in table 1.

Table 1. Fracture Efficacy In Randomised Patients 80-100 Years

	STRONTIUM RANELATE STUDIES					RISEDRONATE 5mg STUDIES				
Fracture Risk	Placebo	Strontium Ranelate	Relative Risk Reduction (p value)	ARR	NNT	Placebo	Risedronate	Relative Risk Reduction (P Value)	ARR	NNT
Vertebral Fracture			59% (p=0.002)	4.8%	21	10.9%	2.5%	81% (p < 0.001)	8.4%	12
1 Year	8.3%	3.5%								
-3 Years	26.5%	19.1%	32% (p=0.013)	7.4%	14	24.6%	18.2%	44% (p= 0.003)	6.4%	16
Non Vertebral Fracture			41% (p=0.027)	2.8%	36	NA	NA	NA	NA	NA
1 Year	6.8%	4.0%						NS	2.2%	-
3 Years	19.7%	14.2%	31% (p=0.011)	5.5%	18	16.2%	14.0%	(p=0.66)	(NS)	(NS)
Hip Fracture			41% (NS	2.2%	-			NS*	0.9%	-
3 Years	7.4%	5.2%	p=0.112)	(NS)	(NS)	5.1%	4.2%	(p=0.35)	(NS)	(NS)

*** Includes Hip study patients only - selected on the basis of non-skeletal risk factors for hip fractures rather than established osteoporosis.**

Conclusion: This literature review reinforces the irony that we have the least evidence for fragility fracture reduction in the group at greatest risk who are likely to sustain the greatest potential harm with the greatest risk of disability and mortality and at potentially the greatest cost to society. This calls for better randomised controlled trials to provide better evidence for treatment of this patient group who are likely to place increasing demands on limited per-capita health care resources in future decades.

Disclosures: ***C.A. Inderjeeth****, Servier 1, 2, 3; Sanofi Aventis 1, 2, 3, 4; MSD 1, 3; Eli Lilly 1, 4.*

SA438

Weight and Body Mass Index Predict Bone Mineral Density and Fractures in Women 40 to 59 years. S. N. Morin[1], J. F. Tsang*[2], W. D. Leslie[2]. [1]Medicine, McGill University, Montreal, QC, Canada, [2]Medicine, University of Manitoba, Winnipeg, MB, Canada.

Risk factors for the prediction of osteoporosis and fractures have not been extensively characterized in younger women. We evaluated the associations between weight, body mass index (BMI), Osteoporosis Self-Assessment Tool (OST), bone mineral density (BMD) and fracture risk in women aged 40 to 59 years.

Using administrative databases, we conducted a retrospective cohort study in 8,254 women age 40-59 years who had baseline BMD testing. Cox proportional hazards models were created to examine the associations with weight, BMI, OST, BMD and subsequent non-traumatic fractures during 3.3 years of follow up.

Body weight, BMI and OST had similar overall performance in their ability to classify women with femoral neck T-score ≤ -2.5 (area under the ROC curve 0.76; 95% CI: 0.73-0.78, 0.72; 95% CI: 0.70-0.75, 0.77; 95% CI: 0.75-0.79, respectively). During 27,256 person-years of observation, 225 women (2.7 %) experienced one or more incident fractures. After adjustment for age, prevalent fractures and use of systemic corticosteroids, each standard deviation decrease in weight was associated with a 19% increase in the risk of incident fracture (95% CI: 1.01-1.35). Femoral neck BMD and the presence of prevalent fractures were also positively associated with the risk of incident fractures.

Low weight and BMI predict osteoporosis and are associated with an increase in the risk of fractures in younger women. The negative impact of low body weight on bone health should be more widely recognized.

Table Risk of Incident Fractures

	Univariate HR (95% CI)	Multivariate HR (95% CI) *without BMD*	Multivariate HR (95% CI) *with BMD*
Model 1			
Weight per SD decrease	1.17 (1.01-1.35)	1.19 (1.03-1.37)	0.97 (0.84-1.14)
Age per decade increase	--	1.07 (0.94-1.22)	0.98 (0.85-1.12)
Prevalent osteoporotic fracture	--	3.28 (2.36-4.57)	2.68 (1.91-3.76)
Systemic corticosteroid use	--	1.24 (0.75-2.04)	0.97 (0.59-1.61)
Femoral neck BMD per SD decrease	--	--	1.61 (1.37-1.89)
Model 2			
BMI per SD decrease	1.17 (1.01-1.35)	1.18 (1.02-1.37)	1.00 (0.86-1.17)
Age per decade increase	--	1.07 (0.94-1.22)	0.98 (0.86-1.12)
Prevalent osteoporotic fracture	--	3.26 (2.35-4.54)	2.70 (1.93-3.78)
Systemic corticosteroid use	--	1.25 (0.76-2.06)	0.98 (0.59-1.63)
Femoral neck BMD per SD decrease	--	--	1.59 (1.36-1.86)
Model 3			
OST per SD decrease	1.19 (1.03-1.38)	1.20 (1.04-1.39)	0.98 (0.83-1.14)
Prevalent osteoporotic fracture	--	3.29 (2.36-4.57)	2.68 (1.91-3.75)
Systemic corticosteroid use	--	1.24 (0.75-2.03)	0.97 (0.59-1.62)
Femoral neck BMD per SD decrease	--	--	1.60 (1.37-1.88)

Disclosures: ***S.N. Morin****, Merck 1; Amgen 4; Novartis 1, 4; Alliance Better Bone Health 1, 4.*

SA439

See Friday Plenary number F439.

SA440

Obesity and Bone Architecture in Men - Can We Apportion the Metabolic and the Mechanical Effect? P. Szulc, S. Boutroy*, P. D. Delmas. INSERM 831, Hopital E. Herriot, Lyon, France.

Obesity is associated with higher areal bone mineral density (aBMD), but architectural basis and pathophysiological mechanisms underlying this association at the weight-bearing and the non-weight-bearing sites are not fully understood. High resolution peripheral quantitative computed tomography was carried out in 1128 men aged 20 to 88 years at the distal radius and distal tibia by using the XtremeCT device (Scanco Medical AG). Architectural parameters were compared in three groups of men according to their body mass index (BMI): normal weight (≤25 kg/m^2, n=349, mean age - 56 years), overweight (25<BMI≤30 kg/m^2, n=555, mean age - 65 years) and obesity (>30 kg/m^2, n=224, mean age - 68 years). For the comparisons between the skeletal sites, all the investigated variables were transformed into Z-score using the parameters of the normal weight men as the reference group. At the radius and tibia, total cross-sectional area did not differed across the groups. After adjustment for age, overweight and obese men had significantly higher cortical area and thickness, higher total and trabecular (but not cortical) volumetric BMD (vBMD), higher trabecular number (but not the estimated trabecular thickness) and more homogenously distributed trabeculae (lower variability of the trabecular separation in an individual). In the overweight and obese men, total, trabecular and outer (subendocortical) trabecular vBMD were similarly increased at the radius (0.25 to 0.39 SD, p<0.005-0.0001) and at the tibia (0.31 to 0.45 SD, p<0.001-0.0001) compared with the normal men. In the overweight and obese men, trabecular separation and its variability were similarly reduced at the radius (0.27 to 0.50 SD, p<0.005-0.0001) and the tibia (0.23 to 0.58 SD, p<0.01-0.0001). By contrast, in comparison with the normal weight men, some parameters were more increased in the obese men at the tibia than at the radius: cortical area (0.61 vs 0.39 SD, p<0.02), trabecular number (0.84 vs 0.62 SD, p<0.03) and the central (inner) trabecular vBMD (0.42 vs 0.26 SD, p<0.03).

Thus, in these predominantly trabecular skeletal sites, obesity was associated with a similar increase in certain parameters at the weight-bearing distal tibia and at the non-weight-bearing distal radius suggesting that they may depend mainly on the metabolic changes related to the obesity e.g., higher peripheral estrogen synthesis. By contrast, in the obese men, the cortical area, the trabecular number and the inner trabecular vBMD were more increased at the weight-bearing distal tibia compared with the non-weight-bearing distal radius suggesting that, in men, the mechanical load may be a significant determinant of these architectural parameters.

Disclosures: ***P. Szulc****, None.*

SA441

A High-fat Diet Negatively Regulates Bone Development in Growing Mice. L. Wang*[1], S. Yan*[2], C. Li*[1], J. Zhao*[3], K. Ding*[4], M. Wang*[5], J. Xu*[1], L. Zhou*[6], M. Hamrick[7], C. Isales[4], Q. Mi[6]. [1]Center for Biotechnology and Genomic Medicine, Medical College of Georgia, Augusta, GA, USA, [2]Dept. of Medicine, The Medical School Hospital of Qingdao University, Qingdao, China, [3]Dept. of Medicine, Shangdong University Medical School, Jinan, China, [4]Dept. of Medicine, Medical College of Georgia, Augusta, GA, USA, [5]Dept. of Physiology, Medical College of Georgia, Augusta, GA, USA, [6]Dept. of Pathology, Medical College of Georgia, Augusta, GA, USA, [7]Dept. of Cellular Biology, Medical College of Georgia, Augusta, GA, USA.

High fat diet (HFD) results in obesity, which is well known to affect both bone density and quality in adults. However, it is unclear how HFD affects the bone development during childhood. The purpose of this study was to determine the effect of HFD on bone structure and mechanics in young adult mice. Female CD1 mice were fed with either HFD or normal fat diet (NFD) starting at 4-weeks of age for 10 weeks. The HFD contained 36% fat (15.2% saturated, 20.8% unsaturated) and NFD 4.4% fat (2.5% saturated, 1.9% unsaturated). The bone mineral content (BMC), bone mineral density (BMD), fat and lean mass were examined in 14-week old mice using dual-energy X-ray absorptiometry, and bone biomechanical properties were also evaluated using three-point bending test. The body weight and fat mass in HFD-treated mice increased almost 2-5 fold compared to that in NFD-treated mice, respectively. The whole body BMD, BMC, bone area and lean mass in HFD-treated mice were slightly higher than that in NFD mice (P>0.05). However, the spine BMC and bone area in HFD mice were significantly lower than that in NFD mice (P<0.01), while femoral BMD (P<0.01), BMC (P<0.01) and bone area (P<0.05) in HFD mice were significantly greater than that in NFD mice. But, there was no statistically different in bone biomechanical values between the two groups. When these results were adjusted based on body weight or fat mass, the body BMD, BMC, bone area, and femur BMD and BMC tended to be lower in HFD-treated mice in comparison to NFD mice although the difference was not statistically significant. Our preliminary data suggest that the vertebral bone is more sensitive to HFD-induced bone loss compared to the long bones in young, growing animals. Considering that childhood and adolescence are crucial periods to acquirer nearly half of the peak bone mass, high-fat diet in young adult might be one reason for the increasing prevalence of osteoporosis during adult and aging.

Disclosures: ***Q. Mi****, None.*

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SA442

Effects of Diacylglycerol Oil on Body Composition, Bone Mineral Density and Bone Strength in Mice. H. Choi*[1], W. Jung*[2], Y. Rhee[1], S. Kim*[3], J. Y. Jeon*[4], I. Paik*[2], E. Lee*[1], S. Lim[1]. [1]Department of Internal Medicine, Yonsei University College of Medicine, Seoul, Republic of Korea, [2]Department of Physical Education, Yonsei University, Seoul, Republic of Korea, [3]Department of Internal Medicine, Kwandong University College of Medicine, Goyang, Republic of Korea, [4]Department of Sports and Leisure Studies, Yonsei University, Seoul, Republic of Korea.

Background: A high fat diet (HFD) is known to promote obesity and insulin resistance, causing metabolically deleterious effects on the body. Total fat intake, especially saturated fat intake, has been reported to be associated with a higher risk of osteoporotic fractures. Meanwhile, consuming a diet rich in diacylglycerol (DAG) oil instead of triacylglycerol (TAG) has been reported to have metabolically beneficial effects such as reduction in body weight and abdominal fat and improvement in lipid profile. However, there has been no study investigating the effects of DAG on bone mineral density (BMD) and strength in mice.

Methods: Four-week-old male 38 C57BL/6J mice were divided into 4 groups: the chow diet group (n=10), HFD group (n=9), HFD with high DAG group (n=10), and HFD with exercise group (n=9). After 17 weeks of treatment, body composition and BMD were measured using DXA, and bone strength of the femur was measured by a 3-point bending test.

Results: Body weight was significantly lower in the HFD with high DAG (38.3 ± 2.0 g) and HFD with exercise (36.2 ± 2.8 g) groups than the HFD group (46.2 ± 2.3 g). Percent fat mass was significantly lower in the HFD with exercise group (32.7 ± 5.6%) than the HFD

group (42.7 ± 3.3%). The HFD with high DAG (0.0668 ± 0.0041 g/cm^2) and HFD with exercise (0.0684 ± 0.0053 g/cm^2) groups had significantly higher femur BMD than the HFD group (0.0611 ± 0.0032 g/cm^2), although such differences were not found in the 3-point bending test.
Conclusions: DAG may have beneficial effects on BMD. Further studies are needed to elucidate the effects of DAG on the skeleton.

***Disclosures: H. Choi**, None.*

SA443

Vitamin K Treatment for One Year Does Not Alter Femur Geometry in Postmenopausal Women. N. Vallarta-Ast[1], D. Krueger[1], J. W. Suttie*[2], N. Binkley[1]. [1]Osteoporosis Clinical Research Program, University of Wisconsin, Madison, WI, USA, [2]University of Wisconsin, Madison, WI, USA.

A potential role of vitamin K in reducing osteoporotic fracture risk remains controversial. Recently, it has been reported that vitamin K supplementation favorably alters femur geometric parameters. Such effects could lead to a reduction in fracture risk but not be appreciated by bone mineral density as measured by dual energy x-ray absorptiometry (DXA). To investigate this possibility, we re-analyzed femur DXA scans from a prospective randomized clinical study of healthy postmenopausal women without osteoporosis. Briefly, in this study, 381 postmenopausal women were randomly assigned to receive phylloquinone (K1) 1,000 mcg daily, menatetrenone (MK4) 45 mg daily, or placebo for one year. Subjects were [mean (± SE)] 62.3 (± 0.4) years and had a mean BMI of 28.5 (± 0.3) kg/m^2. All participants received daily calcium with vitamin D. DXA scans were performed using a GE Lunar DPX-IQ at baseline and after 6 and 12 months. For this report, the baseline and 12 month femur scans were re-analyzed with software version 4.7. Hip geometric parameters were obtained using the software auto-analysis feature and include femur neck and total femur area, femur neck width, hip axis length (HAL), femur neck cross-sectional area (CSA), femur neck cross-sectional moment of inertia (CSMI) and the GE proprietary femur strength index (FSI.)
No change in any of the measured femur geometric parameters was observed in the placebo group and no differences in any parameter were observed with either K1 or MK4 treatment compared with placebo (Table).
Table: Selected femur DXA parameters

Group	N	FN BMC (g)		FN Width (mm)		CSA (mm^2)		FSI	
		Base	12m	Base	12m	Base	12m	Base	12m
Placebo	124	4.342 (.059)	4.343 (.060)	31.28 (.24)	31.39 (.23)	154.2 (2.2)	153.7 (2.2)	1.29 (.03)	1.31 (.03)
K1	115	4.464 (.062)	4.445 (.062)	31.57 (.21)	31.51 (.21)	159.6 (2.3)	159.4 (2.2)	1.43 (.04)	1.45 (.04)
MK4	119	4.432 (.063)	4.407 (.062)	31.72 (.22)	31.72 (.22)	157.8 (2.3)	157.3 (2.3)	1.40 (.03)	1.39 (.03)

Data presented as mean ± SE.
In conclusion, no effect of one-year treatment with vitamins K1 or MK4 on DXA-measured parameters of femur geometry was observed. It remains possible that longer duration studies could identify vitamin K-related changes.

***Disclosures: N. Vallarta-Ast**, None.*

SA444

See Friday Plenary number F444.

SA445

Goettingen Minipigs - A Model for Ca/Vit D-Deficiency Osteomalacia and Steroid-induced Osteoporosis. K. E. Scholz-Ahrens*[1], C. C. Glüer[2], W. Timm*[2], Y. Açil*[3], W. Yan-Classen*[1], J. Schrezenmeir*[1]. [1]Physiologie und Biochemie, Max-Rubner-Institut, Kiel und Karlsruhe, Germany, [2]Medizinische Physik, Klinik für Diagnostische Radiologie, Universitätsklinikum Schleswig-Holstein - Campus Kiel, Kiel, Germany, [3]Klinik für Mund-, Kiefer- und Gesichtschirurgie, Universitätsklinikum Schleswig-Holstein - Campus Kiel, Kiel, Germany.

Ca and Vit D intake of the elderly in Western societies is often inadequate and their Vit D synthesis and renal Ca reabsorption tend to be diminished. To study this condition, we developed a minipig model for osteomalacia by evaluating the effect of Vit D and Ca deficiency in comparison with a model for osteoporosis induced by glucocorticoids. Three diet groups, each made up of ten 30 month-old minipigs, were studied: 1) 0.6% Ca, 6500 IU Vit D/kg diet (control group), 2) 0.2% Ca, no Vit D (osteomalacia); 3) 0.6% Ca, 6500 IU Vit D/kg diet and a daily dose of 1mg/kg BW prednisolone for 8 wks, thereafter 0.5mg/kg BW (osteoporosis). After 15 months, the Ca balance (mg/7d) had decreased in both experimental groups, by 6175 mg (+/-2355) in the osteomalacia group ($p<0.05$), and by 3411(+/-2023) mg in the osteoporosis group ($p>0.05$), while there was only a small decrease of 388 (+/-1146) mg in the control group. The decrease in bone mineral density (BMD, mg/cm3) in vivo was similar in both experimental groups (51.2 +/-14.7 and 56.7 +/-8.3, respectively), and significantly different from the marginal decline in the control group (2.3 +/-11.8). In the osteomalacia group, plasma concentrations of 25(OH)-Vit D were lower ($p<0.05$) and of 1,25$(OH)_2$-Vit D higher ($p<0.05$) than in the osteoporosis and control group. Bone alkaline phosphatase levels tended to be higher in the osteomalacia group, compared to the control, and were significantly higher most of the time than in the osteoporosis group. Bone resorption, evaluated by deoxypyridinoline-crosslinks, was unchanged and not different between groups. Because bone density loss was similar in both conditions, but differed with regard to osteoblast activity and Vit D metabolism, the minipig seems to be a valid model to study Ca/Vit D-deficiency osteomalacia and steroid-induced osteoporosis.

***Disclosures: K.E. Scholz-Ahrens**, None.*
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SA446

Effects of Potassium Bicarbonate on Calcium- and Bone Metabolism during High Salt Intake. P. Frings-Meuthen*, J. Buehlmeier*, N. Baecker*, M. Heer. Institute of Aerospace Medicine, German Aerospace Center, Cologne, Germany.

We have recently shown that high salt intake led to decreases in blood pH and bicarbonate concentration accompanied by increases in bone resorption markers (Frings-Meuthen et al. J Bone Miner Res 2008; 23:517-524). Since pH decrease is a mandatory condition to activate osteoclasts, we hypothesize that the increased bone resorption is initiated by a low-grade metabolic acidosis. Administration of an alkali salt may counteract the acidosis and thereby decrease the bone resorption induced by a high salt intake. In a metabolic ward study with 8 healthy male test subjects (mean age: 25.8 ± 3.9 years; body weight (BW) 75.4 ± 3.6 kg) we examined the ability of $KHCO_3$ to prevent salt-induced bone loss. 4 days of adaptation with a normal salt intake (2.8 mmol/kgBW/d) were followed by 10 days of high salt intake (7.7 mmol/kgBW/d) combined in one trial with a supplementation of 3 x 30 mmol $KHCO_3$ per day in a cross-over design. Urinary calcium (UCa) excretion and bone resorption markers (C- and N-terminal telopeptide of type I collagen (CTX, NTX)) were analyzed in all 24-hour urine collections. Fasting morning blood was analyzed for the bone formation markers, bone specific alkaline phosphatase (bAP), and N-terminal of propeptide of type I procollagen (PINP). Postprandial parathyroid hormone (ppPTH) measurements were used to provide an insight into the involvement of PTH in salt-induced bone loss. $KHCO_3$ supplementation was able to reduce salt-induced calciuria by only 12 % ($p = 0.04$) and NTX excretion by only 8 % ($p = 0.04$); no significant decrease was observed in the bone resorption marker CTX ($p = 0.18$). Bone formation marker PINP ($p = 0.92$) and bAP ($p = 0.95$) remained unchanged. However, ppPTH levels were twofold higher 25 minutes after $KHCO_3$ supplementation ($p < 0.001$). High salt intake itself did not affect ppPTH. The $KHCO_3$-induced PTH increase might play an important role in the attenuated effectiveness of potassium bicarbonate to reduce salt-induced bone loss.

***Disclosures: P. Frings-Meuthen**, None.*

SA447

Ovariectomy-Induced Hyperphagia Does Not Modulate Bone Mineral or Bone Strength in Female Sprague-Dawley Rats. J. M. Y. Jiang*, S. M. Sacco*, W. E. Ward. Nutritional Sciences, University of Toronto, Toronto, ON, Canada.

Osteoporosis is a skeletal disorder characterized by compromised bone strength, leading to an increased susceptibility to fragility fractures. The adult ovariectomized (OVX) rat is the FDA-recommended model for postmenopausal osteoporosis and plays a crucial role in the development of prevention and treatment strategies. While ovariectomy closely mimics the postmenopausal skeleton, it also induces secondary effects of hyperphagia, increased weight gain, and adiposity. Increased body weight is associated with protective effects on bone, and thus may be a confounder in preclinical studies investigating the effects of interventions on bone health. For this reason, pair-feeding is commonly used to control food intake and prevent hyperphagia-associated weight gain. However, the effects of hyperphagia on bone and the importance of pair-feeding have not been elucidated. The objective of this study was to determine whether the type of feeding, pair versus *ad libitum*, modulates bone mineral and bone strength using the OVX rat model of postmenopausal osteoporosis. Three-month old female Sprague-Dawley rats (n=14/group) were randomized to 1) sham-operated control (SHAM); 2) ovariectomized pair-fed (OVX-PF); or 3) ovariectomized *ad libitum* (OVX-AL). All rats were fed a standard pelleted diet (AIN93M). Pair-feeding involved matching the food intake of OVX-PF rats to the average amount of food eaten by the SHAM group the day before. At the end of 14 weeks, lean mass and fat mass were measured by dual energy x-ray absorptiometry (DEXA). Bone mineral density (BMD) and biomechanical bone strength were assessed at the femur and the lumbar vertebrae (LV). OVX-AL rats consumed more food ($P<0.05$) than both the SHAM and OVX-PF during the first 5 weeks of study, but hyperphagia disappeared by 7 weeks. Final body weight ($P<0.01$), weight gain ($P<0.01$), and fat mass ($P<0.05$) were higher among OVX-AL rats than both SHAM and OVX-PF rats, while no differences were observed between the SHAM and OVX-PF groups. SHAM rats had higher femur ($P<0.001$) and LV1-3 BMD ($P<0.001$) than both the ovariectomized groups. The peak load of LV4 was also significantly higher ($P<0.01$) among the SHAM rats compared to the ovariectomized groups. No differences in bone outcomes were observed between OVX-PF and OVX-AL groups. In summary, ovariectomy-induced hyperphagia and weight gain do not modulate BMD or biomechanical bone strength at 14 weeks post-ovariectomy. Pair-feeding is not necessary to prevent excess weight gain and adiposity from confounding bone outcomes in this model.

***Disclosures: J.M.Y. Jiang**, None.*
This study received funding from: Discovery Grant from the Natural Sciences and Engineering Research Council of Canada to W. Ward.

SA448

High-fat Diet Facilitated Decreases in BMD of Ovariectmized Mice and Suppressed the Anabolic Effects of PTH on Bone. S. Tanaka[1], A. Sakai[1], T. Tanigawa*[2], H. Hirasawa*[1], Y. Katae*[1], K. Tanaka*[1], T. Nakamura[1]. [1]Orthopaedic Surgery, University of Occupational and Environmental Health, Kitakyushu, Japan, [2]1st medicine, University of Occupational and Environmental Health, Kitakyushu, Japan.

The post-menopausal osteoporosis is caused by decreases in estrogen activity and sequential increases in bone turnover. High-fat diets also decreased bone volume, what we clearly demonstrated in our recent study using mouse given high-fat diet due to increases in bone resorption and decrease in bone formation. We hypothesized that decreases in BMD of estrogen deficient women is facilitated by high-fat diet.
We tested the effects of high-fat diet to the ovariectomized (OVX) mice on bone metabolism and of intermittent administration of PTH on recovery from decreased bone. Seven weeks old, female, C57BL/6J mice were purchased and subjected to 6 groups, sham operated mice given standard chow, OVX mice given standard chow, sham given high-fat diet, OVX given high-fat (OH), sham given high-fat diet and intermittent PTH injection, and OVX given high-fat and PTH, after 1 week acclimatization and operated. Feeding started from 8 weeks age and 3 times per one week PTH injection were performed from 14-week (6 weeks after operation) until the end of the experiment
Decreases in BMD were observed in the lumbar spine of OVX mice and what was more apparent in OVX mice given high-fat diet, while the lumbar BMD did not decreased in sham operated mice given high-fat diet. The PTH injection partially recovered lumbar BMD up to the OVX mice given standard chow level. In femur, although BMD in OVX mice given standard chow or sham operated mice given high-fat diet did not decrased, BMD in OVX mice given high-fat diet decreased apparently. PTH injection recovered the femoral BMD in OVX mice given high-fat diet to the levels of sham operated or OVX mice given standard chow.
High-fat diet facilitated decreases in BMD of OVX mice and suppressed the anabolic effects of PTH on bone especially in non-weighted site.

Disclosures: ***S. Tanaka****, None.*

SA449

Treadmill Exercise Provides Only Short-Term Protection Against Cancellous Bone Loss with Reduced Dietary Energy Intake: Endocrine Mechanisms. S. N. Swift[1], K. Baek[1], M. J. De Souza[2], S. A. Bloomfield[1]. [1]Intercollegiate Faculty of Nutrition, Health & Kinesiology, Texas A&M University, College Station, TX, USA, [2]Kinesiology, Pennsylvania State University, University Park, PA, USA.

Dietary energy deficits leading to weight loss can induce loss of cancellous bone. We sought to elucidate whether endurance exercise maintains cancellous bone mass in female rats subjected to a 40% energy deficit. Forty Sprague-Dawley virgin female rats 5-mo-old were acclimated to AIN-93M purified diet for 8 wks. For the next 12 wks, the control groups (ADLIB-EX & ADLIB-SED) were fed AIN-93M *ad lib*. Energy restriction with exercise group (ER-EX) and sedentary energy-restriction (ER-SED) groups were fed modified AIN93M diet with 30% and 40% less energy, respectively, with 100% of all other nutrients provided. Exercising rats performed treadmill running 4 d/wk, 90-100 min/d (~60% V_{O2} max) to increase weekly energy expenditure by 10%. At day 0, wk 4, and wk 12, urine & serum were collected for ELISA assays on IGF-1, estrogen glucuronide (E1G), leptin, C-terminal telopeptides of type I collagen (CTx) and N-terminal propeptides of type I collagen (P1NP). At the same time points, peripheral quantitative tomography (pQCT) scans were performed at the proximal tibia metaphysis (PTM) for cancellous volumetric bone mineral density (vBMD. Body weights for all *ad lib* fed animals increased by 12 wk (+9%) while all ER rats lost weight (-16%), with no effect of exercise in either energy status group. ER decreased cancellous vBMD (-17%) by 12 wk in sedentary animals. Exercise increased cancellous vBMD by 4 wk regardless of energy status (+12%), but did not protect against losses by 12 wk in ER-EX (-9%). Bone turnover appears to be suppressed in both ER groups, with decreases in P1NP (-43%) and CTx (-4%) by 12 wk, but not in ADLIB-EX. Serum leptin declines significantly in EX-ER by 4 wk (-75%) and in SED-ER at 12 wk (-96%). Trends were observed for suppression of serum IGF-1 in both ER groups that were significant in ER-EX (-33%) by 12 wk. Uterine weight was significantly lower in ER-EX at 12 wks (-48% vs. ADLIB-EX). In summary, endurance training in energy-replete rats increased cancellous vBMD and protected against bone loss in rats experiencing energy deficits at 4 wk, but not when restriction was maintained for 12 wk. Estrogen status (estimated by uterine weight) and endocrine markers of energy status were maintained in ADLIB-EX rats, but declined over time in all energy-restricted rats (SED and EX). Energy deficit-induced changes in estrogen status, leptin and IGF-I may contribute to this loss of cancellous bone.

Disclosures: ***S.N. Swift****, None.*
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SA450

Prevention of Glucocorticoid-induced Osteoporosis with Alendronate or Alfacalcidol in Patients with Ophthalmologic Diseases. T. Ikeda*[1], C. Hamanishi*[1], S. Souen*[2]. [1]Orthopaedic Surgery, Kinki Univ. School of Medicine, Osaka, Japan, [2]Orthopaedic Surgery and Rheumatology, Kinki Univ. Nara Hospital, Nara, Japan.

Glucocorticoid (GC)-induced osteoporosis is a major public health problem for patients taking GC therapy. To clarify the drug therapy strategy for the increased risk fracture, we studied the changes in bone mineral density (BMD) by dual energy X-ray absorptiometry after initiating high dose GC treatment over 12 months in patients with ophthalmologic diseases. Alendronate or alfacalcidol treatment was started concurrently with GC treatment. We studied 36 Japanese patients (12 male and 24 female) suffering from ophthalmologic diseases who required long-term (>3 months) treatment with oral GC at an average daily dose of at least 10 mg prednisolone.
The mean BMD in alfacalcidol group were continuously lower than it's baseline during 12 months treatment. The lowest mean changes of BMD were observed at 9 months in both alendronate group (-1.14%) and for alfacarcidol group (-5.01%).
In male subjects, the mean changes of BMD for alendronate group were greater than for alfacalcidol group at 3 months and 6 months after treatment. In female alfacalcidol group, significant BMD reductions from baseline were observed in comparison to female alendronate group at both 3 and 6 months. In addition, we checked urinary excretion of type I collagen cross-linked N-telopeptides corrected for creatinine (NTx/Cr) data as a marker for bone turnover. For alendronate group, the mean percentage changes from baseline were -41.7% and -42.3% at 3 and 6 months after treatment, respectively. For alfacalcidol group, the mean percentage changes from baseline were 11.3% and -4.4% at 3 and 6 months after treatment, respectively. In alendronate group, the mean decrements of NTx/Cr were statistically greater in comparison to alfacalcidol group.
In conclusion, we have investigated the preventive effect of alendronate or alfacalcidol for GC-induced osteoporosis in patients with ophthalmologic field diseases. Alendronate is efficacious in preventing bone mass at the lumber spine in above populations. In addition, alfacalcidol might be efficacious in the restricted population such as male subjects as a second-line anti-osteoporotic agents.
In this study, the enrolled population includes relative young female population and also includes male patients, and the comparison between postmenopausal patients and premenopausal patients might be important, therefore, we should design further studies to solve these points.

Disclosures: ***T. Ikeda****, None.*

SA451

Effects of Intermediate Doses of Glucocorticoids on Bone Turnover and Circulating Dkk-1, MIF, sRANKL and OPG in Patients with Interstitial Lung Disease. A Three-Month Longitudinal Study. A. Dovio*, E. Palmas*, L. Saba*, A. Termine*, E. Bianco*, L. Mercante*, C. Albera*, A. Angeli. Dept. of Clinical and Biological Sciences, University of Turin, Orbassano - Torino, Italy.

High-dose glucocorticoid (GC) administration causes early and transient increase of bone resorption. Whether such an increase occurs also for intermediate and low doses is unknown. Dynamics over time of this effect, relationships to routes of administration and schedules of tapering down doses, and underlying mechanisms are unclear. We have performed a longitudinal study lasting a 3-month span in patients diagnosed as having interstitial lung disease responsive to GCs and starting treatment with intermediate doses.
Fourteen patients (M/F 8/6; age 54, 34-77 yr (median, range)) suffering from sarcoidosis (n=10), nonspecific interstitial pneumonia (n=2), usual interstitial pneumonia (n=1), desquamative interstitial pneumonia (n=1) were included. All patients did not take drugs affecting bone, including GCs, in the previous 6 months. Serum osteocalcin (OC), C-telopeptide of type I collagen (CTX), Dickkopf-1 (Dkk-1), osteoprotegerin (OPG), total soluble RANKL (sRANKL), and macrophage migration inhibitory factor (MIF) were measured by commercially available ELISAs at baseline, after 3 days and after 1, 2, 4, 8, 12 weeks of treatment.The starting dose was 25 (n=3) - 50 mg (n=11) of prednisone; the dose at week 12 was 12.5 (5-25) mg; cumulative dose and average daily dose were 1925 (1225-2975) mg and 23 (15-35) mg, respectively. With this regimen, GCs caused a rapid decrease of serum OC (P<0.001 by multiple measures ANOVA - at day 3 median -34%, P<0.001 vs baseline by Dunnett's test), persistent up to week 2, with a subsequent progressive raise until week 12; levels comparable to baseline, however, were not attained. CTX showed a marginally significant trend to an early increase (P=0.10 by ANOVA), peaking at day 7 (median +28%, P=0.02 vs baseline by Dunnett's test) and declining thereafter. Consistently, OC/CTX ratio showed a rapid, marked and sustained decrease (P<0.001). Serum OPG showed an early and persistent decrease (P<0.01 by ANOVA - at day 7 median -19%, P=0.03 vs baseline by Dunnett's test). No significant effect of GC therapy was noticed on serum sRANKL, MIF and Dkk-1. Our data indicate that also a regimen of intermediate doses of GCs with rapid tapering down is able to alter in a few days the balance between bone resorption and formation. They lend support to the preventive use of antiresorptive drugs in patients starting such regimens. Less availability of OPG in the bone microenvironment deserves attention among mechanisms accounting for the early increase of bone resorption.

Disclosures: ***A. Dovio****, Procter & Gamble 2; Stroder 5; Novartis 5; Eli-Lilly 5.*

SA452

See Friday Plenary number F452.

SA453

Impaired Angiogenesis and Compromised Fluid Volume Accompanies Increased Osteoblast and Osteocyte Apoptosis with Glucocorticoid Excess: Interconnected Pathogenetic Changes Responsible for the Loss of Bone Strength. Q. Liu*[1], C. Wan[1], Y. Wang[1], E. A. Hogan*[2], S. B. Berryhill*[2], S. C. Manolagas[2], T. L. Clemens[1], R. S. Weinstein[2]. [1]Department of Pathology, University of Alabama at Birmingham, Birmingham, AL, USA, [2]Center for Osteoporosis and Metabolic Bone Diseases, University of Arkansas for Medical Sciences and Central Arkansas Veterans Healthcare System, Little Rock, AR, USA.

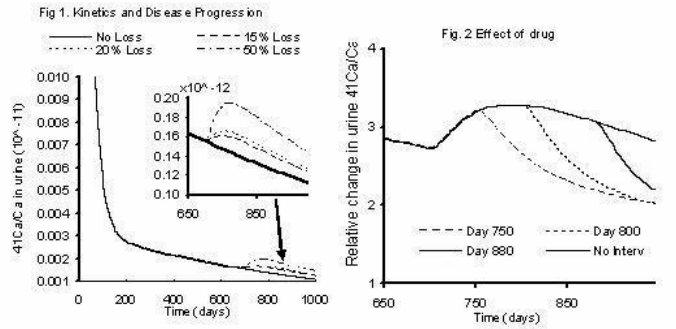
Glucocorticoid (GC) excess causes a decline in bone strength that is greater than the loss of bone mass, but the mechanisms behind these phenomena remain unknown. Based on evidence that glucocorticoids may affect blood flow as well as the vasculature itself, we have undertaken in vivo and in vitro studies to elucidate the effects of GCs on bone fluid and vasculature in the mouse and the cellular and molecular mechanism responsible for such effects. After 28 days of administration of prednisolone to 8-month-old C57Bl/6 mice, vertebral and femoral BMD decreased by 4.5-6.6% ($p < 0.003$), osteoblast and osteocyte apoptosis increased by 160-250% ($p < 0.01$), while vertebral compression strength and femoral 3-point bending decreased by 25% ($p < 0.04$). These changes were associated with a striking decrease in interstitial fluid, as determined by 2-dimensional fluorescent imaging of the osteocyte-lacunar-canalicular system using Procion Red-a metabolically inert tracer. Further, prednisolone decreased vertebral vessel volume and surface area by 79-74% ($p < 0.05$), as determined by 3-dimensional microCT imaging following lead chromate perfusion. The changes in fluid and vasculature volume were associated with diminished immunostaining for the endothelial markers CD34 and von Willebrand factor-factor VIII complex in cancellous bone sections. In agreement with the in vivo data, dexamethasone (DEX) (1, 10, 100 nM) impaired endothelial sprouting as assessed by CD31 immunostaining in fetal metatarsals from both wild type mice and mice overexpressing VEGF. Likewise, DEX dose dependently (1 nM-1 μM) impaired tube-like structure formation of cultured human umbilical vein endothelial cells (HUVECs). At higher doses (1 μM), DEX induced HUVEC apoptosis as determined by increased caspase 3/7 activity ($p < 0.05$). At 10 and 100 nM, DEX decreased BrdU positive cell numbers, while at a low dose (1 nM), modestly increased BrdU positive cell numbers. Collectively, these results strongly suggest that decreased osteocyte-lacunar-canalicular fluid volume, diminished bone vasculature, increased osteoblast and osteocyte apoptosis, and impaired angiogenesis represent interconnected pathogenetic changes that are responsible for the disproportional loss of bone strength over bone mass with glucocorticoid excess.

***Disclosures:* *C. Wan**, None.*

SA454

Development of Empirical Model Using ^{41}Ca for the Study of Bone Remodeling in Human. M. Sharma*, S. K. Hui. Department. of Therapeutic Radiology, University of Minnesota, Minneapolis, Minneapolis, MN, USA.

Osteoporotic fractures are a significant public health problem, resulting in substantial morbidity and mortality. Initial studies show that the ^{41}Ca assay may become useful diagnostic tool for monitoring metabolic bone disease and its treatment management (1, 2). There is lack of empirical model to study the effect of bone loss and antiresorptive therapy on ^{41}Ca based bone turnover assay. The purpose of this study is to (a)develop a general empirical model that can describe ^{41}Ca tracer kinetics in human;(b)study its feasibility to monitor metabolic bone disease and (c)monitor response to preventive drugs.
A mathematical model has been developed using the Berkeley software to study calcium kinetics and investigate bone turnover. Difference in the contributions of oral and intravenous drug is also taken into account. The rate of bone loss has been parameterized to detect percent change in bone turnover with the severity of disease. The change in ^{41}Ca/Ca from steady state during metabolic bone disease causing bone loss is calculated over time. Bone dynamics after bisphosphonate drug intervention is simulated and verified.
Our model based on four compartments fits well with the available experimental data (1,2). Figure 1 and its inset reflects the bone loss due to severity of disease by urine calcium ratio increase over time compared to the steady state. The relative effect of bisphosphonate intervention at 15% bone loss is shown in Figure 2. Early intervention (day 750) with drug shows early recovery of bone remodeling compared to late intervention (day 880).



The present ^{41}Ca tracer kinetics model will be a useful tool to understand how ^{41}Ca assay monitor bone remodeling is affected by disease progression and drug intervention. This may allow better treatment management to prevent bone fracture and will be a useful guide in the use of ^{41}Ca assay for clinical scenarios.

References:
1. E. Denk et al., J. Bone Miner. Res. 22(10):1518, 2007 and references therein.
2. S.K. Hui et. al., Nucl. Inst. and Methd. in Phys.Res. B 259(1):796, 2007 and references therein.

***Disclosures:* *M. Sharma**, None.*
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SA455

See Friday Plenary number F455.

SA456

Impact of Physical Inactivity (10 Days Bed Rest) on Markers of Bone Turnover in Young Men with Low and Normal Birth Weight. K. Karnik[1], D. Talbot*[1], J. Dick*[1], M. Sonne*[2], L. Højbjerre*[2], A. Alibegovic*[3], B. Stallknecht*[2], F. Dela*[2], A. Vaag*[3]. [1]Corporate Research, Unilever plc, Bedfordshire, United Kingdom, [2]University of Copenhagen, Copenhagen, Denmark, [3]Steno Diabetes Centre, Copenhagen, Denmark.

Physical activity plays an important role in maintaining skeletal integrity throughout life. Reduced skeletal loading, as often seen in the elderly as a result of long term bed rest and reduced mobility, has been shown to result in bone loss. It has been proposed that low birth weight is associated with changes in adult body composition including low bone mineral content. In this study, we compare the effect of bed rest for 10 days on the bone turnover markers in young men with low and normal birth weight.
40 healthy men aged 21-28 yrs were recruited in the study, 20 of which were low birth weight (LBW) individuals born at term and 20 were normal birth weight (NBW) individuals born at term. Participants were confined to a hospital bed for 10 days and consumed a standardised diet. At the end of the intervention period, all the participants underwent 4-weeks of rehabilitation training programme under supervision.
At the end of the intervention, serum carboxy-terminal collagen crosslink (CTX) increased by 31.6% in the entire group, which was statistically significant ($p<0.0001$). The mean percent increase from baseline for serum CTX for the LBW group was 37% and for NBW group was 28%. Although, the differences between LBW and NBW groups for percent change in CTX did not reach the level of statistical significance, there was a trend for higher bone resorption as a result of bed rest in the LBW group. At the end of bed rest period, serum parathyroid hormone decreased significantly ($p<0.001$) in the entire group. The mean percent decrease was 24% in the LBW group and 23% in NBW group. The difference between the groups was not statistically significant. There was no difference in the levels of serum osteocalcin as a result of bed rest.
These results confirm the detrimental effect of bed rest on the skeletal health as evident by the increase in serum CTX. Our findings also suggest a trend for greater loss of skeleton in the LBW group as a result of bed rest. This particular finding needs to be investigated further with larger number of participants.

***Disclosures:* *K. Karnik**, Unilever plc 5.*

SA457

Influence of Markers of Bone Turnover on Calcaneal Quantitative Ultrasound: Results from the European Male Ageing Study (EMAS). S. R. Pye*[1], T. W. O'Neill[1], H. Borghs*[2], D. Vanderschueren[2], J. E. Adams[3], K. A. Ward[3], G. Bartfai*[4], F. Casanueva*[5], J. D. Finn*[6], G. Forti*[7], A. Giwercman*[8], I. T. Huhtaniemi*[9], K. Kula*[10], M. Punab*[11], A. J. Silman*[1], F. C. Wu*[6], S. Boonen[2]. [1]ARC Epidemiology Unit, The University of Manchester, Manchester, United Kingdom, [2]Katholieke Universiteit Leuven, Leuven, Belgium, [3]Department of Imaging Science and Biomedical Engineering, The University of Manchester, Manchester, United Kingdom, [4]University of Szeged, Szeged, Hungary, [5]University of Santiago de Compostela, Santiago de Compostela, Spain, [6]Department of Endocrinology, The University of Manchester, Manchester, United Kingdom, [7]University of Florence, Florence, Italy, [8]Lund University, Malmo, Sweden, [9]Imperial College, London, United Kingdom, [10]University of Lodz, Lodz, Poland, [11]University of Tartu, Tartu, Estonia.

The majority of studies on biochemical measurements of bone turnover have focused on women with fewer data in middle aged and elderly men. The aim of this analysis was to characterize the influence of bone markers on calcaneal ultrasound measurements in middle aged and elderly European men.
Men aged between 40 & 79 years were recruited from population registers in 8 European centres. Subjects were invited to attend for quantitative ultrasound (QUS) of the calcaneus (Hologic - SAHARA) and a fasting blood sample from which the bone markers serum N-terminal propeptide of type 1 procollagen (P1NP) and crosslinks (-Ctx) were measured. In three of the recruitment centres, osteocalcin (OC) and serum C-terminal telopeptide of type 1 collagen (ICTP) were also measured. Height and weight were assessed in all subjects. The relationships between QUS parameters (broadband ultrasound attenuation [BUA] and speed of sound [SOS]) and bone markers were assessed using linear regression with adjustments made for age, height, weight and centre.
3136 men, mean age 60.0 years (standard deviation [SD]=11.0) were included in the analysis. Mean BUA was 80.1 dB/MHz (SD=19.0) and SOS 1550.6 m/s (SD=34.1). Mean P1NP was 41.6 ng/ml (SD=17.6), OC 22.1 ng/ml (SD=7.1), -Ctx 353.6 pg/ml (SD=181.8) and ICTP 3.2 ng/ml (SD=0.9). There was a significant decrease in OC levels with age (β= -0.066; p<0.01) and an increase in ICTP (β= 0.024; p<0.001). P1NP and -Ctx were unrelated to age. Higher -Ctx levels were associated with lower BUA (β = -0.006; 95% CI -0.010, -0.003) and SOS (β = -0.016; 95% CI -0.022, -0.009). Higher OC levels were associated with lower BUA (β = -0.159; 95% CI -0.304, -0.014) while higher P1NP levels were associated with lower SOS (β = -0.114; 95% CI -0.180, -0.048). ICTP levels were not associated with either QUS parameter.
In this population survey of middle aged and elderly European men, higher levels of bone turnover markers were associated with lower QUS parameters.

***Disclosures: S.R. Pye**, None.*
This study received funding from: Commission of the European Communities.

SA458

See Friday Plenary number F458.

SA459

Bone Loss With Smoke Exposure and Lrp5 Expression. G. K. Alvarez, B. T. Hackfort*, M. P. Akhter, J. A. Yee, D. M. Cullen. Osteoporosis Research Center, Creighton University, Omaha, NE, USA.

Smoking is a risk for osteoporosis associated with low bone mass and poor fracture healing, but the mechanism for these effects is unknown. This study examined the interaction of Lrp5 receptor (low density lipoprotein like receptor protein) and smoke exposure on bone. Lrp5 is a Wnt co-receptor and the G171V mutation in LRP5 (HBM) is a gain of function mutation with greater WNT/β catenin signaling that results in high bone mass.
We examined bones after 12 wks of smoke exposure in WT (C57Bl6), Lrp5+/-, and HBM female (5.3 ± 0.5mo) and male mice. Mice were randomly assigned to control or smoke exposure (3 hrs/d 5 d/wk at 129±38 mg/mm^{3}TSP). Female femora (15/grp) were processed for cortical and cancellous histomorphometry. Measurements included area (total, marrow, bone volume -BV/TV), mineralizing surface (MS/BS), mineral apposition rate (MAR), and bone formation rate (BFR/BS). Male tibiae (8/grp) were cleaned, frozen at -80C, mRNA extracted, and mRNA quantified relative to control (ABI 7500, control GAPDH). Differences due to smoke and genotypes were analyzed by GLM. There were no differences in initial body weight (25 ±3 g), but controls gained more weight than treated mice by 12 wks. In cortical bone there was no difference due smoke exposure in total area, but marrow area was greater in Wt and HBM mice than control mice (table). Exposure to smoke increased periosteal BFR in the Lrp5 +/- mice, but not in WT and HBM mice. There were no effects on endocortical BFR. Cancellous BV/TV was significantly lower in HBM mice after smoke exposure, but not in WT or Lrp5+/- mice where initial BV/TV was <3%. MS/BS was depressed in WT mice and tended downward in the other genotypes. Osteoclast surface was lower in the HBM animals than the other genotypes, but there was no smoking effect. Analysis of WNT responsive genes showed that Cyclin D1, associated with cell proliferation, was suppressed with smoke exposure in Wt (0.6 ±0.1) and Lrp5+/- (0.7±0.1) mice, but not HBM mice (1.0 ±0.1). PTGS2 was elevated in response to smoke exposure in HBM (3.2 ±1.2), but not in Lrp5+/- (1.8 ±0.8) or WT (0.5 ±0.1) mice.
Smoke exposure resulted in cortical and cancellous bone loss especially in HBM mice, but HBM cancellous BV/TV remained 10 fold greater than in WT and Lrp5+/- bone. Bone turnover reached a steady state after 12 wks of treatment. While the HBM gain of function mutation maintained or increased gene expression for the WNT responsive genes tested, this did not prevent bone loss. \

	Cortical Midshaft Femur			Cancellous Distal Femur		
Treatments	Mar Area (mm^2)	P-BFR (µm/yr)	E-BFR (µm/yr)	BV/TV (%)	MS/BS (%)	OcS/BS (%)
Lrp5+/- con	0.77 (0.06)	28 (17)	98 (63)	1.6 (1.2)	23 (9)	6.8 (3.7)
smoke	0.80 (0.05)	48 (25) [a]	113 (41)	1.2 (2.0)	20 (8)	7.5 (5.0)
Wt con	0.80 (0.07)	49 (18)	127 (43)	2.7 (1.0)	27 (6)	8.7 (5.2)
smoke	0.87 (0.06) [a]	34 (16)	101 (40)	1.1 (0.7)	20 (8) [a]	8.5 (4.9)
HBM con	0.76 (0.11)	44 (22)	101 (32)	22 (8)	25 (8)	4.6 (3.4)
smoke	0.87 (0.10) [a]	30 (15)	86 (23)	14 (6) [a]	24 (7)	4.2 (3.7)
Int (Gene x Trt)	ns	0.004	ns	0.003	ns	ns

Mean (S.D.) [a] smoke effect P<0.05

***Disclosures: G.K. Alvarez**, None.*
This study received funding from: Nebraska Department of Health and Human Services.

SA460

Profile Analysis of Rodent Metaphyseal Trabecular Bone Reveals a Biphasic Dose-Dependent Response To Administered Bone Active Agents. A. Pitsillides[1], P. L. Salmon[2], A. Tivesten[3], S. Moverare-Skrtic[3], C. Ohlsson[3], L. Oste[4], G. Dixon[4], A. Idris[5], R. Van T'Hof[5]. [1]Royal Veterinary College, London, United Kingdom, [2]SkyScan, Kontich, Belgium, [3]Sahlgrenska Institute, Goteborg, Sweden, [4]Galapagos plc, Mechelen, Belgium, [5]Molecular Medicine Centre, Edinburgh University, Edinburgh, United Kingdom.

The rodent bones in the vicinity of the knee - the proximal tibia and distal femur - are standard sites for bone morphometric analysis for disease models such as ovariectomy-induced osteoporosis. There are two ways in which the amount of metaphyseal trabecular bone can change: either by a change to the balance of formation and resorption at the surface of the trabecular bone throughout the metaphysis, or, in young rodents, by a change to the rate of output of secondary trabecular bone from the growth plate and primary spongiosa.
Here we review three rodent OVX studies involving ovariectomy and drug treatment of (initially) 2-3 month old rodents, all employing micro-CT imaging. The 3D images of the whole metaphyses are reanalysed to assess the profile of trabecular percent crossectional area (BA/TA) with increasing distance from the growth plate - the “metaphyseal profile” - in order to elucidate the relative roles of these two above mechanisms in bringing about change in metaphyseal trabecular volume. These studies include treatment with the sex steroids estrogen and testosterone, the bisphosphonate alendronate, PTH and the novel antiresorptive ABD365.
The results suggest a biphasic dose-dependent effect of treatment. Where trabecular volume in OVX rodents is restored by administered treatment to levels similar to or just above sham control levels, the first mechanism is predominant, the alteration of remodeling balance throughout the metaphysis, causing uniform elevation of the metaphyseal profile but no change to its slope. However where a treatment causes trabecular volume to be elevated to a level substantially above sham control levels, an upturn in the metaphyseal profile occurs in the metaphyseal region closest to the growth plate, implicating the additional effect of a second mechanism, that of an increased rate of new trabecular bone output at the growth plate.
Sex steroids and alendronate are both demonstrated to elevate trabecular volume to well above the sham level in a manner involving this latter mechanism. This indicates that even generally anti-resorptive compounds such as the bisphosphonate alendronate, at high enough dose levels, can stimulate increased growth plate formation of trabecular bone.

***Disclosures: P.L. Salmon**, SkyScan 3.*
This study received funding from: SkyScan.

SA461

See Friday Plenary number F461.

SA462

Trabecular Bone Loss in Response to Transient Muscle Paralysis Is Not Gender Dependent. T. S. Gross, S. Srinivasan, B. J. Ausk*, S. L. Poliachik*, S. D. Bain. Orthopaedics and Sports Medicine, University of Washington, Seattle, WA, USA.

Whether gender provides systemic influences that significantly impact disuse induced bone loss remains controversial. In general, however, the female skeleton is thought to be more sensitive than the male skeleton to bone loss observed in models such as hindlimb suspension. In this study, we sought to clarify this relation in an in vivo model in which focal osteoclastogenesis is induced by transient muscle paralysis. High resolution (11 micron) microCT images of the proximal tibia metaphysis and lower resolution images of the entire lower limb (21 micron) were obtained for 7 female and 7 male C57B6 mice (16 wk) immediately prior to injection of Botox into the calf muscle group (2U/100 g, d 0). In vivo scans of all mice were also obtained by d 7 and d 15 post post induction of muscle paralysis. Standard image analysis procedures were used to determine trabecular bone parameters within the proximal tibia, while the lower resolution imaging was used to assess soft tissue volume changes. All data were assessed by comparison with time zero data. Lower limb soft tissue volume was significantly decreased by 7 d (F: -11.8 ± 3.5%, M: -11.5 ± 1.9%) and further decreased by 15 d (F: -24.2 ± 7.3%, M: -21.8 ± 3.5%; all $p < 0.01$), with no gender based differences. Within 7 d, proximal tibia BV/TV was significantly diminished in both female (-61.7 ± 10.2%, p=0.02) and male mice (-44.4 ± 6.4%, p=0.02). The loss of BV/TV in female mice was significantly greater than that of male mice (p=0.03). Further BV/TV degradation was observed by 15 d but the magnitude of bone loss was equal across gender (F: -70.2 ± 9.3%; M: -70.6 ± 6.9%, both $p<0.01$). At both time points, degraded BV/TV was associated with a significant erosion of trabecular thickness, decreased trabecular number, and increased trabecular spacing. No gender based statistical differences were identified for any of these morphological parameters. In summary, we found that female mice demonstrated a more rapid loss of BV/TV within the first 7 d than male mice, but the extent of bone trabecular degradation was equivalent across gender by 15 d. Whether this transient difference arose due to gender associated levels of basal bone turnover will require further clarification. In our view, the equivalent degradation of muscle volume across gender and equivalent deterioration of trabecular architecture within 15 d suggests that the signaling pathway responsible for rapidly initiating osteoclastogenesis following transient muscle paralysis is the same in females and males. These results also suggest that gender based differences previously observed in other disuse models may be associated with acute osteoblastic responses identified in those models.

Disclosures: ***T.S. Gross****, None.*

SA463

Brain-derived Serotonin Positively Regulates Bone Mass Accrual. V. K. Yadav*, F. Oury*, J. Ryu*, N. Suda*, P. Ducy*, G. Karsenty. Genetics and Development, Columbia University, New York, NY, USA.

Serotonin (5-hydroxytryptamine) is a biogenic amine that functions both as a neurotransmitter in mammalian central nervous system and as a hormone in the periphery where most of (~95%) it is produced. Remarkably serotonin does not cross the blood-brain barrier thus it has potentially two different functions depending on its site of synthesis. Serotonin synthesis is initiated following tryptophan hydroxylation by an enzyme called tryptophan hydroxylase (Tph). Brain-derived serotonin synthesis is initiated by Tph2 and peripheral-derived serotonin is initiated by Tph1. To analyse the spatio-temporal pattern of serotonin expression and the function of brain-derived serotonin on bone mass, we introduced a *LacZ* allele in the mouse *Tph2* locus by homologous recombination in embryonic stem cells. LacZ staining during embryonic development and postnatally delineated serotonergic neurons in the mouse brain and showed that in the adult, Tph2 neurons are clustered in to six groups in the brain stem, those neuronal groups are known as dorsal, ventral and caudal raphe nuclei. Furthermore, histological analysis of the vertebrae and long bones revealed that *Tph2*-deficient mouse develop a severe low bone mass phenotype early during adult life thus identifying serotonin as the first bona fide neuropeptide regulating bone mass. Histomorphometric analysis revealed that this is due to a decrease in bone formation and an increase in bone resorption. We have also identified through genetic means one of the 14 serotonin receptor that is responsible for the regulation of bone mass. We will present at the meeting the signaling pathway in which brain-derived serotonin acts.

Disclosures: ***V.K. Yadav****, None.*

SA464

Kenney-Caffey Syndrome in a Caucasian Man. P. Tondapu*[1], C. V. Odvina[2]. [1]Mineral Metabolism, UT Southwestern Medical Center, Dallas, TX, USA, [2]Charles and Jane Pak Center for Mineral Metabolism, UT Southwestern Medical Center, Dallas, TX, USA.

29 year old white man presented to our Mineral Metabolism Clinic with history of hypocalcemia, hypomagnesemia, short stature and hypoparathyroidism. He was born full term by spontaneous vaginal delivery, weighing 6 lb 11oz. There is no family history of consanguinity. He developed generalized tonic clonic seizures about 1 week after birth and was found to have hypocalcemia. Initial diagnosis was pseudohypoparathyroidism and he was managed with calcium supplements. Subsequent evaluation revealed papilledema. Cranial sutures fused at age 4. At age 12 years and 10 months, he had a bone age of a 14 year old and at age 13 1/2 years; he was -3.14 SD (29kg) for weight and -3.22 SD (134 cm) for height. Additional work up showed low calcium, magnesium and PTH but normal somadomedin C and IGFBP 3 which ruled out growth hormone deficiency. He has poor vision all his life and is legally blind without glasses but has no hearing problem. When he was seen at our clinic, it was noticed that he has short stature (135 cm), brachydactyly and facial dysmorphism with microcephaly, micrognathia, deep set eyes, hypotelorism, beaked nose and poor dentition with malformed tooth. He has had multiple fractures related to trauma and poor union in one of them. He has not been taking calcium regularly and has had multiple admissions for hypocalcemia. Work up showed normal serum magnesium, low calcium and PTH. Fractional excretion of magnesium was 2.7%, which ruled out renal magnesium wasting as the cause of hypomagnesemia. DEXA scan showed high Z score (+3.2) at the spine and slightly low Z scores at the hip(-0.5) and distal 1/3 of the radius (-1.7). Karyotype is normal male (46XY). Because of the clinical findings, we suspected Kenny-Caffey syndrome and DNA was sent for genetic testing to confirm the diagnosis. Kenney-Caffey syndrome is an extremely rare hereditary skeletal disorder characterized by osteosclerotic bone dysplasia with associated hypocalcemia, dental caries and ocular abnormalities. Kenney-Caffey and related disorder HRD map to 1q42-q43 and are due to mutations in the tubulin specific chaperone E (TBCE) gene, a protein involved in tubulin folding. Most of the cases that had been described in the literature were of Arab decent. To the best of our knowledge, there is only one reported case of this syndrome in a Caucasian patient.

Disclosures: ***P. Tondapu****, None.*

SA465

See Friday Plenary number F465.

SA466

Bony Symptoms and Vertebral Fractures in Gaucher Disease Type 1. Data from the 105 Patients of the French Observatoire on Gaucher Disease. R. Javier*[1], R. Jaussaud*[2], C. Rose*[3], P. Chérin*[4], E. Noël*[5], C. de Roux Serratrice*[6], D. Dobbelaere*[7], A. Hartmann*[8], E. Hachulla*[9], B. Grosbois*[10], C. Roux[11]. [1]Rheumatology, Universitary Hospital, Strasbourg, France, [2]Internal Medicine, Hôpital Robert Debré, Reims, France, [3]Haematology, Hôpital St Vincent de Paul, Lille, France, [4]Internal Medicine, Pitié-Salpêtrière, Paris, France, [5]Internal Medicine, Universitary Hospital, Strasbourg, France, [6]Internal Medicine, Hôpital St Joseph, Marseille, France, [7]Paediatry, Universitary Hospital, Lille, France, [8]Neurology, Hôpital Pitié-Salpêtrière, Paris, France, [9]Internal Medicine, Hôpital Claude Huriez, Lille, France, [10]Internal Medicine, Hôpital Sud, Rennes, France, [11]Rheumatology, Hôpital Cochin, Paris, France.

Background/objectives: Gaucher disease type1 (GD1) is a rare disease with a heterogeneous clinical presentation. A French national prospective registry was implemented to describe the clinical features of adult GD1 patients with focus on bone symptoms and their impact on patients'quality of life.
Methods: Clinical data were collected during a routine visit. No additional tests were performed. A specific case report form was designed to guide the physicians.
Results: From March 2005 to September 2006, 105 patients were included and represent about one third of the French GD1 population. Mean age (SD) was 45 (14) years (54% female). Early diagnosis (< 1 year after the first signs) was less frequent when first signs are skeletal manifestations (25% vs 47%). 85% of patients had a history of bony symptoms including osteonecrosis in 30 %and 17% of patients had undergone joint replacement at a mean age (SD) of 40 (10) years. Other manifestations are bone infarction 28 %, bone crisis 21 %, peripheral fracture 18 % and vertebral fractures (VF) 15% (to be compared to 9-11% in the literature). Almost all vertebrae can be involved, from T3 to L5, and many patients have 2 to 4 concomitant VF. Most of these VF, even those of grade 3 according to Genant classification, occurred spontaneously or for minimal trauma at a mean age of 43 years (min-max: 29-61). Chronic back pain remained in half of these patients. Of the 64 patients with bone mineral density data available, 44% had osteopenia and 18% had osteoporosis.
Conclusion: These results illustrate the high frequency of bone fragility in GD1 patients and the frequency of VF. Bone disease remains a major issue in GD1 patients and a multidisciplinary team approach including bone evaluation and specific bone fragility treatment seems essential for optimizing patients'care.

Disclosures: ***R. Javier****, None.*
This study received funding from: Actelion.

SA467

Age-Related Changes in Bone Mineral Density, Cross-Sectional Area and the Strength of Rat Femur; an Involvement of Suppression of Growth Hormone-IGF-1 Signaling. M. Tomita[1], S. Motokawa*[2], H. Shindo*[1], K. Kumagai*[1], I. Shimokawa*[3]. [1]Orthopedic Surgery, Nagasaki University Graduate School of Medicine, Nagasaki, Japan, [2]Orthopedic Surgery, National Nagasaki Medical Center, Ohmura, Japan, [3]Investigative Pathology, Nagasaki University Graduate School of Medicine, Nagasaki, Japan.

In order to investigate aging-related changes in the bone structure and strength and an involvement of the growth hormone (GH)-insulin-like growth factor (IGF)-1 axis, we examined bone mineral density (BMD), cross-sectional area (Area), and bone strength strain index (SSI) of rat femoral bone by peripheral quantitative computed tomography (pQCT).
Materials and Methods: We used wild type (WT) male Wistar rats and the transgenic rat strain (mini), in which GH secretion was suppressed by overexpression of anti-sense GH gene. F1 hybrid rats (F1), which showed intermediate phenotypes in the GH-IGF-1 level and body weight between WT and mini rats, were also used. Rats were sacrificed at 6, 15, and 24 months of age (mo). The cancellous bone parameters of BMD, Area, and SSI were measured at 12mm proximal side from growth plate of distal femur by pQCT.
Results: 1) WT showed the highest BMD of total, subcortical bone, and cancellous bone at 6, 15, and 24 mo. F1 showed the intermediate values of BMD between WT and mini rats at 6, 15, and 24 mo. Cancellous bone showed the most apparent age-dependent decrease in all groups.
2) Areas of total, subcortical bone, and cancellous bone at 6, 15, and 24 mo were highest in WT. And F1 showed the intermediate value of those three groups at 6, 15, 24 mo. Area continued to increase between 6 and 24 mo in WT, but in F1 and mini Area it increased from between 6 and 15 mo, but not thereafter.
3) All SSI's (X-, Y-, and polar-) showed almost same age-dependent change as was in Area. WT showed the highest values of SSI in 6, 15, and 24 mo. In WT, SSI continued to increase between 6 and 24 mo, but in F1 and mini, SSI increased between 6 and 15 mo, but decreased thereafter.
Disscussion: Age-dependent changes were the most obvious in cancellous bone BMD in all groups. Area and SSI showed almost same age dependent-changes but those were different from those of BMD. These data suggested that bone strength correlated not only to BMD, but also Area. Senile bone frailty might cause the changes of bone morphology. Suppression of GH secretion decreased all of BMD, Area, and SSI. GH secretion level plays a role in maintenance of the bone quality during the aging.

***Disclosures:* M. Tomita**, *None.*

SA468

Adult Hypophosphatasia Treatment with Teriparatide. P. M. Camacho[1], A. M. Mazhari*[1], R. Kadanoff*[2]. [1]Endocrinology, Loyola University Medical Center, Maywood, IL, USA, [2]Rheumatology, Loyola University Medical Center, Maywood, IL, USA.

Hypophosphatasia is a rare inherited metabolic bone disorder that is characterized by defective bone mineralization due to a deficiency of tissue-nonspecific alkaline phosphatase (TNSALP), which results from a deactivating gene mutation. Inorganic phosphate accumulates extracellularly impairing bone mineralization.
A 68-year-old African-American female was evaluated at the Loyola University Osteoporosis and Metabolic Bone Disease Center in April 2006 for low bone mass and low serum total alkaline phosphatase (AP). She reported a femur fracture at age 27 after a fall and a metatarsal fracture in her 50's. She was treated with prednisone since 2002 (ranging from 10-60 mg/day) for her dermatomyositis. She had a maternal history of fractures. Other medical illnesses included hypertension, Reynaud's, reflux disease and pulmonary fibrosis. The patient was on esomeprazole, ranitidine, methotrexate, tacrolimus (for dermatomyositis), furosemide and calcium carbonate. She had been on ergocalciferol 50,000 international units/wk and oral ibandronate 150 mg for 6 months prior to her initial visit.
The diagnosis of hypophosphatasia was based on the following biochemical findings and clinical history: persistently low AP ranging from 11-20 U/L (30-110 U/L), low bone specific alkaline phosphatase (BSAP) ranging from 2.9-3.4 ug/L (7-22 ug/L), high pyridoxal 5'-phosphate (PLP) 95 ng/mL (5-30 ng/mL) and history of low-trauma fractures. Her baseline urinary N-telopeptide on ibandronate was 32 nm BCE/mm creat (26 - 124). She had a normal serum creatinine, ionized calcium, phosphorus, intact PTH and 25 OH-Vitamin D and 24-urine calcium. Mutation analysis for TNSALP gene is pending.
Her DXA scan in 2005 showed lumbar spine bone mineral density (BMD) of 1.006 gm/cm2, T-score -1.6, Z-score -1.4; mean femoral neck BMD of 0.693 gm/cm2, T-score -2.9 and Z-score -2.8. There was a significant decline of 14% in the hip and 13% in the spine and compared to the 2003 DXA.
In May 2006, ibandronate was discontinued and four months later, BSAP remained low at 2.9 ug/l. Teriparatide 20 ug subcutaneously was given. BSAP subsequently increased to 5 U/L at 7 months. After 13 months of teriparatide therapy, BSAP increased to 7 U/L, AP increased from 14 to 26 IU/L and PLP decreased from 95 to78 ng/ml.
Her follow up DXA in October of 2007 (spine BMD 1.012 gm/cm2, T-score -1.6 and mean femoral neck BMD 0.714 gm/cm2, T-score -2.3) showed stable BMD of the lumbar and femoral neck regions. She did not report any fractures during the treatment period.
Our results showed beneficial effects of teriparatide on adult hypophosphatasia which is similar to two previously reported cases. Teriparatide is a promising agent for this metabolic bone disease.

***Disclosures:* R. Kadanoff**, *None.*

SA469

See Friday Plenary number F469.

SA470

Clinical Usefulness of Measurement of Fibroblast Growth Factor 23 (FGF23) in Hypophosphatemic Patients-Proposal of Diagnostic Criteria using FGF23 Measurement. I. Endo[1], S. Fukumoto[2], K. Ozono[3], N. Namba[3], H. Tanaka[4], D. Inoue[5], M. Minagawa[6], T. Sugimoto[7], M. Yamauchi[7], T. Michigami[8], T. Matsumoto[1]. [1]Medicine and Bioregulatory Sciences, University of Tokushima Graduate School of Medical Sciences, Tokushima, Japan, [2]Division of Nephrology & Endocrinology, Medicine, University of Tokyo Hospital, Tokyo, Japan, [3]Department of Pediatrics, Osaka University Graduate School of Medicine, Osaka, Japan, [4]Pediatrics, Okayama Saiseikai-Sogo Hospital, Okayama, Japan, [5]Third department of Medicine, Teikyo University School of Medicine, Chiba, Japan, [6]Pediatrics, Chiba University Graduate School of Medicine, Chiba, Japan, [7]Endocrinology/Metabolism and Hematology/Oncology, Shimane University School of Medicine, Shimane, Japan, [8]Bone and Mineral Research, Osaka Medical Center for Maternal and Child Health, Osaka, Japan.

Fibroblast growth factor 23 (FGF23) plays central roles in the development of hypophosphatemic diseases such as tumor-induced osteomalacia (TIO) and X-linked hypophosphatemic rickets/osteomalacia (XLH). However, clinical usefulness of FGF23 measurement has not been established. The objective of this study is to validate FGF23 measurement and establish diagnostic criteria for these diseases. In this cross-sectional study, we examined serum intact-FGF23 concentrations by two-site IRMA (Kainos, Japan) and other biochemical parameters of phosphate metabolism in 32 patients with TIO, 28 patients with XLH and 16 hypophosphatemic patients with other causes including vitamin D deficiency, Fanconi's syndrome and Cushing's syndrome. These patients were recruited by questionnaires to members supported by grants from Research on Measures for Intractable Diseases by Japanese Ministry of Health, Labor and Welfare. In patients with TIO, serum phosphate was 1.67 +/- 0.08 mg/dl with severely suppressed TmP/GFR (1.24 +/- 0.08) and high FGF23 (1149 +/- 541 pg/ml). In patients with XLH, basically the same findings were observed with serum FGF23 being 155 +/- 30 pg/ml. FGF23 was above the upper limit of the reference range in most patients even under medical treatment with active vitamin D3 and/or phosphate. The lowest FGF23 in these patients was 38.0 pg/ml. In contrast, FGF23 in hypophosphatemic patients with other causes was undetectable in 12 out of 16 patients and the highest FGF23 in this group was 23.9 pg/ml. These results clearly indicate that FGF23 measurement is useful for the differential diagnosis of hypophosphatemic diseases caused by excess FGF23 actions and other etiologies. We propose that the intact FGF23 of more than 30 pg/ml in hypophosphatemic patients indicates the presence of diseases caused by FGF23.

***Disclosures:* I. Endo**, *None.*

SA471

See Friday Plenary number F471.

SA472

Vitamin D Deficiency in Inner City Infants: Impact of DBP Genotype. T. O. Carpenter[1], J. H. Zhang*[2], F. Herreros*[1], E. Torrealba-Fox*[1], C. Simpson*[3], M. Savoye*[1], N. Held*[1], B. Ellis*[1], D. E. C. Cole[4]. [1]Pediatrics (Endocrinology), Yale University School of Medicine, New Haven, CT, USA, [2]The Cooperative Studies Program Coordinating Center, VA Connecticut Healthcare System, West Haven, CT, USA, [3]Internal Medicine, Yale University School of Medicine, New Haven, CT, USA, [4]Laboratory Medicine and Pathobiology, Medicine, and Genetics, University of Toronto, Toronto, ON, Canada.

The prevalence of nutritional rickets is not known but is thought to occur more frequently in northern latitudes and in urban settings. Biochemical abnormalities are detectable before overt skeletal disease is seen. To ascertain risk for development of nutritional rickets in a northern latitude urban environment, we sampled over 500 healthy infants (6 mos - 3 yrs old) during well-child visits to neighborhood clinics in our area. Demographics and detailed diet histories (3 separate 24-hr recall interviews) were obtained. Serum Ca (10.1 ± 0.5 mg/dl), P (5.4 ± 1.7 mg/dl), alkaline phosphatase (AP, 322 ± 453 IU/L), PTH 22 ± 19 nlEq/ml), 25-OHD (25D, 27 ± 6 ng/ml), and 1,25(OH)2D (1,25D, 66 ± 24 pg/ml) were measured and genotypes for VDR, TRPV6, and DBP were analyzed. Average daily intakes of calcium (745 ± 299 mg), phosphate (772 ± 280 mg), and protein (39 ± 15 g) were calculated (NDS, U. Minn.).
Prevalences of hypovitaminosis D (25D < 20 ng/ml), hyperparathyroidism, and elevated AP were 12.9%, 24.4%, and 12.6 % respectively. 3.5% of subjects had elevations in both PTH and AP. 25D correlated negatively with PTH (R = -0.098, P = 0.02), but not significantly with AP. In patients with normal PTH, 25D was 27 ± 6 ng/ml. In a preliminary genetic analysis, DBP D432E genotype correlated closely with both 25D (levels lowest in DD genotype, which was less than DE, which was less than EE, P = 0.003) and 1,25D (highest in DD, which was greater than DE and EE, P = 0.02). T436K also correlated with 25D (levels in TT greater than TK and KK, P = 0.018) and 1,25D (TT greater than TK,

which was greater than KK, P = 0.037). Multivariate regression adjusting for age and season indicates that both DBP loci are significant (P < 0.002) predictors of 1,25D, while D432E is the stronger predictor (P = 0.02) of 25D. Analysis of VDR and TRPV6 genotypes were unrevealing.
This study suggests a lower than expected "risk" for rickets based on the limited number of combined biochemical abnormalities observed. These data provide useful population reference data for 25-OHD levels in the pediatric population where vitamin D deficiency is often suspected, and for which age-specific normative data is rarely provided for interpretation. Finally there is a significant dependence of both 25D and 1,25D levels on DBP genotype. DBP genotype may impact the interpretation of 25D level as an index of total body vitamin D stores in this population.

***Disclosures:* *T.O. Carpenter**, None.*
This study received funding from: Gerber Foundation.

SA473

See Friday Plenary number F473.

SA474

A Novel Hypothesis Explains the Syndrome of Chronic Musculoskeletal Pain and Comorbid Painful Healed Fracture Sites in Vitamin D Deficiency. T. R. Roesel. Deployment Health Clincal Center, Walter Reed Army Medical Center, Washington, DC, USA.

Vitamin D deficiency was found in 93% of 150 patients from the general population with chronic musculoskeletal pain (Plotnikoff, Mayo Clin Proc. 2003; 78:1463). A retrospective chart review revealed vitamin D deficiency in nearly half (49%) of 61 service members with chronic pain who returned from Iraq and Afghanistan (Roesel, J Occ Environ Med. 2008, in press). Service members were referred over an 18 month period from March 2005 until September 2006 to a program for graded exercise and cognitive behavior therapy, and were screened for vitamin D deficiency. Referral was for treatment of post traumatic stress disorder or multiple unexplained physical symptoms. Vitamin D deficiency was defined as <20 ng/ml. Five of these veterans with chronic musculoskeletal pain and vitamin D deficiency had healed, but still painful, deployment-related fracture sites. Holick (Mayo Clin Proc. 2003 Dec; 78(12):1457) has proposed that vitamin D deficiency associated bone and muscle pain is generated by inadequate mineralization of the endosteal and periosteal bone matrix leading to matrix swelling that stimulates periosteal nerve pain fibers. Since service members also revealed concomitant chronic widespread musculoskeletal pain beyond the localized bone injury, a new alternative and augmentative hypothesis is hereby proposed to explain the phenomenon of chronic pain in the presence of vitamin D deficiency. Recent identification of vitamin D receptors in the hypothalamus and amygdala by Eyles et al. (J Chem Neuroanat. 2005; 29:21), suggests that vitamin D deficiency may impair known descending pain inhibitory pathways that regulate the peri-aqueductal grey matter, which in turn controls ascending pain signals travelling through the spinothalamic tract. Thus, the localized chronic bone pain at the fracture site, as well as the co-morbid chronic musculoskeletal pain beyond the fracture site, can be explained through vitamin D deficiency through these two mechanisms, one at the healed fracture site, and the other within the central nervous system. Anecdotal improvement in generalized and local pain seen with supplementation suggests that further studies are needed to determine to what extent vitamin D supplementation prevents or ameliorates chronic generalized musculoskeletal pain and localized pain at healed fracture sites in this newly defined syndrome.

***Disclosures:* *T.R. Roesel**, None.*

SA475

Large Collaborative Study on Geographic Variation of *SQSTM1* Mutations in Paget's Disease of Bone in Italy. L. Gennari[1], F. Gianfrancesco*[2], M. Di Stefano*[3], D. Rendina*[4], D. Merlotti[1], T. Esposito*[2], V. De Paola[1], A. Aloia*[2], G. Martini[1], M. Mazzetti*[3], S. Gallone*[5], I. Rainero*[5], L. Pinessi*[5], G. Isaia[3], P. Strazzullo*[4], R. Nuti[1], G. Mossetti*[4]. [1]Internal Medicine, Endocrine-Metabolic Sciences and Biochemistry, University of Siena, Siena, Italy, [2]Institute of Genetics and Biophysics, CNR, Naples, Italy, [3]Internal Medicine, University of Turin, Turin, Italy, [4]Clinical and Experimental Medicine, Federico II University of Naples, Naples, Italy, [5]Neuroscience, University of Turin, Turin, Italy.

Paget disease of bone (PDB) is a chronic disease of the skeleton with a consistent genetic component. The geographic distribution of PDB is not uniform, with a higher prevalence of the disease in populations of British descent. Moreover increased prevalence areas have been described in different Countries. We recently characterized an area of increased prevalence of PDB in the region of Campania, in Southern Italy. Patients from this region also showed increased severity of disease with peculiar phenotypic characteristics and an increased number of familial cases. In this study we examined the clinical characteristics, and the prevalence and type of *SQSTM1* mutations in a large sample of 542 unrelated PDB subjects from several regions including 164 patients from Campania. Nine different mutations in *SQSTM1* gene were observed in 35.2% and 11.3% of familial and sporadic PDB cases, respectively (equivalent to 15.6% of the overall cohort). Five of these mutations, M401V, A427D, G425E, Y383X and 1224-1226 insT leading to E396X were novel and have not been previously described. The other mutations, P387L, P392L, M404V and G425R have been previously described. An higher prevalence of *SQSTM1* mutations was observed in polyostotic than monostotic cases (7% vs 22%; p<0.005). The distribution of mutations in this sample was more heterogeneous than in other countries, with a significantly increased prevalence of the M404V. In keeping with previous studies, the P392L was however the most common observed mutation in both sporadic and familial cases. Genotype-phenotype analysis confirmed an increased severity of disease and an earlier age of onset in mutations that insert a stop codon. Interestingly, in PDB subjects from Campania a different distribution and a significantly reduced prevalence of mutations was observed with respect to the other regions, despite an increase in disease severity and the higher prevalence of familial cases. In particular, in this region less than 20% of familial PDB had SQSTM1 mutation, as compared to 36-50% in the other regions. This might imply the presence of mutations in different genes as well an increased persistence of a possible environmental trigger, or both these conditions.

***Disclosures:* *L. Gennari**, None.*

SA476

See Friday Plenary number F476.

SA477

Archaeological Skeletons Support a North-West European Origin for Paget's Disease of Bone. S. Mays. English Heritage, Portsmouth, United Kingdom.

Although the aetiology of Paget's disease of bone (PDB) remains incompletely understood, it seems clear that there is a strong genetic predisposition. This, coupled with the marked geographic variation in prevalence of PDB, with high prevalences in populations of British origin, has led to the suggestion that the disease originated in Britain and was spread around the world by migration and admixture of British populations(1). The aim of the current work is to investigate this suggestion using the geographical distribution of PDB cases in skeletons excavated from archaeological sites.
The methodology for the study is meta-analysis of the world literature on PDB in archaeological skeletons published from 1889 to the present. Re-evaluation of published work indicates that many dubious archaeological cases of PDB have entered the literature, and have been treated in previous reviews as though they were good data. For diagnosis of PDB in archaeological cases to be reliable it needs to be based on currently acceptable radiographic and/or histological criteria. Published examples, and unpublished cases known to the author, that met these criteria were included in the current study.
Using these criteria, 109 archaeological cases of PDB were identified (table 1). The sex ratio was 2.4: 1 in favour of males. The earliest known cases date from the time of the Roman Empire (1st-4th cent. AD). No cases were identified outside western Europe. Ninety-four percent of cases came from England. The British cases show no particular geographical distribution; the largest numbers of cases come from London but this simply reflects the large number of archaeological skeletons excavated there.

	Archaeological cases of PDB			
Country	Males	Females	Unstated sex	TOTAL
France	1	1	0	2
Germany	0	0	3	3
Portugal	1	0	0	1
England	49	20	34	103
TOTAL	51	21	37	109

The results indicate that PDB has existed in western Europe for at least 2000 years. The concentration of archaeological cases of PDB in western Europe, and in Britain in particular, supports the hypothesis that PDB originated in that geographical area.
References
1. Cundy T. et al. 1999. Paget's disease in New Zealand: is it changing? Bone 24 (5): 7S-9S.

***Disclosures:* *S. Mays**, None.*

SA478

See Friday Plenary number F478.

SA479

Efficacy and Safety of Intravenous Zoledronic Acid in the Treatment of Patients with Resistant Paget's Disease of Bone. J. R. Tucci. Medicine, Roger Williams Medical Center, Providence, RI, USA.

Though responses have been seen in many of our patients with Paget's disease (PD) treated with several potent bisphosphonates, not infrequently, remissions and especially sustained remissions have not been induced. Two recent reports have described greater efficacy and more sustained remissions following zoledronic acid (ZA) as compared with risedronate. Objectives of this study were to determine 1) the efficacy of ZA in patients with PD who had not had a remission or had had a relapse within a year of therapy with other bisphosphonates, 2) the duration of remissions, and 3) the safety of ZA. Inclusion criteria include serum alkaline phosphatase (AP) at least 50% greater than the upper limit of normal and creatinine clearance of ≥35 ml/min. Six females and 6 males, mean age 78 years, were studied. At baseline, blood chemistry profile, serum 25-OHD, and urine NTx/creatinine (NTx) were obtained. Patients were supplemented with up to 1.5 g of Ca and at least 600 units of vitamin D daily. Patients received an infusion of 5 mg of ZA. 9 to 11 days later, serum Ca levels were measured. AP and NTx were measured at 1, 3, 6, 9, and 12 months and subsequently at 4 monthly intervals.
Results: At baseline, serum Ca, phosphate, and 25-OHD levels were within normal limits. Mean creatinine clearance was 64 ±3 ml/min. Following therapy, 3 patients had mild flu-like symptoms. Nine to 11 days after therapy, mean serum Ca fell from 9.5±.11 to 8.9 ±.12 mg/dl. Two patients developed asymptomatic hypocalcemia with serum Ca of 8 mg/dl and 8.3 mg/dl. Excess AP levels fell by 72-100% in the 12 patients. AP normalization occurred in 11 patients and in all but one patient within 1 to 6 months. Normalization of NTx was evident in these 11 patients and in 10 within 1 month. The following data in 11 patients in remission (mean ± SE):

	preRx	1 mo	3 mos	6 mos	9 mos	12 mos	16 mos	Normal Range
AP	321±65	166±21	92±7	80±7	77±7	81±6	84±8	25-100
NTx	247±47	32±8	37±10	32±7	37±9	33±5	46±12	19-66

At 16 months, AP and NTx remain normal in 9 patients. At baseline and 6 months, serum 25-OHD levels were 34±0.9 ng/ml and 33±1.5 ng/ml, respectively, and at baseline and 12 months, creatinine clearances were 64±3.4 ml/min and 68±5.5 ml/min, respectively. In summary, in 12 patients with suboptimal responses to previous bisphosphonate therapy, ZA induced a response in all 12 patients with remissions in 11 patients that are continuing at 16 months in 9 patients. Adverse effects were flu-like symptoms in 3 patients and asymptomatic hypocalcemia in 2 patients. These observations in a selected group of patients with resistant PD treated with ZA demonstrate early suppression of pagetic activity, a continuing remission at 16 months in 9 of 12 responders, good tolerability and renal safety.

Disclosures: ***J.R. Tucci****, GlaxoSmithKline 1, 3; Novartis 1, 2, 3; Merck 1; P&G Pharmaceuticals, Inc. 1, 2.*
This study received funding from: Novartis Pharmaceutical Company.

SA480

See Friday Plenary number F480.

SA481

Comparison of Intravenous and Intramuscular Neridronate Regimens for the Treatment of Paget's Disease of Bone. D. Merlotti[1], D. Rendina*[2], G. Mossetti*[2], L. Gennari[1], V. De Paola[1], G. De Filippo*[2], G. Martini[1], A. Avanzati*[1], P. Strazzullo*[2], R. Nuti[1]. [1]Internal Medicine, Endocrine-Metabolic Sciences and Biochemistry, University of Siena, Siena, Italy, [2]Department of Clinical and Experimental Medicine, Federico II University of Naples, Naples, Italy.

Aminobisphosphonates represent the most common treatment for Paget's disease of bone (PDB), with the potential for sustained remission. Intravenous regimens with different compounds demonstrated improved efficacy and compliance with respect to oral regimens. In a recent study we demonstrated that either zoledronate (4 mg) and neridronate (200 mg) given as a single intravenous infusion showed a similar efficacy in achieving biochemical remission at 6 and 12 months in up to 90% of patients non-responders to pamidronate. In this study we compared the effects of a same neridronate dose (200 mg) given as intravenous (i.v.) (100 mg infusion over a 2-hours period for 2 consecutive days) or intramuscular (i.m.) (25 mg once a week for 1 month) regimen in 56 patients with active PDB. Randomization was stratified according to baseline total alkaline phosphatase (ALP) levels and previous bisphosphonate treatment. All patients were advised to receive calcium plus vitamin D supplementation throughout the study period (1 g of calcium and 800 UI colecalciferol per day). Blood samples were collected at baseline and after 3, 6, and 12 months. The primary efficacy end-point was the rate of therapeutic response at 6 months, defined as normalization of ALP levels or a reduction of at least 75% in total ALP excess. Serum levels of bone ALP, C-terminal telopeptides of Type I collagen, and 25-hydroxyvitamin D (25 OH-D) were also measured. A significant 40-50% decrease in ALP levels was observed with both regimens after 3 months. At 6 months, 92% and 96% of patients receiving i.v. and i.m. neridronate, respectively had a therapeutic response. Normalization of ALP levels at 6 months was achieved in 89% of patients in i.v. group and in 93% of patients in i.m. group. Interestingly, the response to treatment was significantly correlated with baseline ALP and 25 OH-D levels at 6 months. The decrease in ALP levels was highest in patients with higher baseline total or bone specific ALP levels and with higher 25 OH-D levels at 6 months. Normalization in ALP levels was maintained at 12 months in 22/27 (81.5%) and 24/29 (83%) of patients, in i.v. and i.m. neridronate groups, respectively. Both regimens were well tolerated. The only side effect was an acute phase response, occurring in 11-13% of the patients. In conclusion, both i.m. and i.v. neridronate regimens (at a cumulative dosage of 200 mg in 12 months) showed a similar efficacy in achieving biochemical remission in up to 90% of patients with active PDB.

Disclosures: ***D. Merlotti****, None.*

SA482

Usefulness of *HRPT2* Gene and Parafibromin Studies in Two Patients with Primary Hyperparathyroidism and Uncertain Pathological Assessment. C. Marcocci[1], E. Pardi*[1], E. Ambrogini*[1], C. Banti*[1], S. Borsari*[1], P. Viacava*[2], A. Pinchera*[1], F. Cetani*[1]. [1]Endocrinology, University of Pisa, Pisa, Italy, [2]Oncology, University of Pisa, Pisa, Italy.

HRPT2 and parafibromin studies improved the diagnostic accuracy in two patients with primary hyperparathyroidism (PHPT) referred to us after parathyroidectomy, in whom the clinical data were at variance with the pathological diagnosis of adenoma and carcinoma, respectively. Patient #1 had had a 1.5-cm tumor easily removed with a histological diagnosis of parathyroid carcinoma and normocalcemia for 2 years. Re-examination of the histology showed no cardinal signs of parathyroid cancer. Patient #2, with severe PHPT, had had the removal of a 3.5-cm tumor described histologically as adenoma. Ten years later PHPT recurred and persisted despite removal of two parathyroid glands that were normal by routine histological examination. Re-review of the initial histology showed a trabecular pattern, fibrous bands, and atypical mitoses, suggesting an atypical adenoma. Because of the suspicion that case #1 could be an atypical adenoma and case #2 a carcinoma furthers molecular studies were performed. No *HRPT2* and parafibromin abnormalities were identified in patient #1, strongly indicating a benign lesion. In patient #2, an *HRPT2* germline mutation was found (E115X in exon 4) and associated with no parafibromin staining. These data, together with the clinical features, supported the suspicion of a parathyroid carcinoma that was confirmed by histological examination of further slides of the tumor, showing capsular and vascular invasion. A lung 1.5-cm nodule detected by CT was excised. Histology showed a metastasis of parathyroid carcinoma.
HRPT2 gene studies may be of clinical utility in the management of patients with parathyroid tumors of uncertain pathology.

Disclosures: ***C. Marcocci****, None.*

SA483

See Friday Plenary number F483.

SA484

Low Density Lipoprotein-Cholesterol Levels Affect Vertebral Fracture Risk in Female Patients with Primary Hyperparathyroidism (pHPT). H. Kaji[1], I. Hisa*[1], Y. Inoue*[1], T. Sugimoto[2]. [1]Kobe University Graduate School of Medicine, Kobe, Japan, [2]Shimane University Faculty of Medicine, Izumo, Japan.

Patients with pHPT have reduced bone mineral density (BMD) with enhanced bone turnover. The risk of vertebral and forearm fractures are increased in pHPT patients. Although several reports indicated that increased arterial stiffness and dyslipidemia were observed in pHPT patients, it still remains unclear about the relationships between lipid and bone metabolism in pHPT patients, especially about fracture risk. The present study was performed to examine the relationship between lipid metabolism parameters including body composition and bone metabolism in 116 female patients with pHPT and 116 age-matched control subjects. BMD and body composition were measured by dual-energy x-ray absorptiometry. Serum levels of total cholesterol (T-Chol) and high density lipoprotein-cholesterol (HDL-Chol) as well as albumin (Alb) were significantly lower in pHPT group, compared with those of control group. Fat mass (FM) and lean body mass (LBM) as well as % fat were similar in both groups. Serum levels of all parameters (T-Chol, LDL-Chol, TG and HDL-Chol) were not significantly related to serum levels of calcium and PTH as well as bone metabolic indices. As for BMD parameters, only serum LDL-Chol levels were significantly and negatively related to z-score of BMD at femoral neck, but not those of BMD at lumbar spine and radius. FM was positively related to Z-score of BMD at lumbar spine and femoral neck. On the other hand, LBM was negatively related to serum levels of PTH and bone resorption indices and positively to Z-scores of BMD at all. Serum levels of T-Chol and LDL-Chol were significantly lower in the group with vertebral fractures, compared with the group without vertebral fractures, in only pHPT patients. Serum levels of HDL-Chol, TG and Alb as well as body composition parameters were not significantly different between the groups with and without vertebral fractures in both control and pHPT groups. In a univariate logistic regression analysis, age, height, BMD at lumbar spine and radius, serum levels of creatinine, T-Chol and LDL-Chol were selected for the predictors of vertebral fracture risk. Multiple logistic regression analyses suggest that the relationship between LDL-Chol and vertebral fractures was independent of these parameters. In conclusion, the present study demonstrated that lower serum LDL-Chol levels were related to vertebral fracture risk independently of renal function, age, body size index, BMD, bone turnover and serum levels of Ca, PTH, Alb in pHPT women. Increased LBM seemed to be protective for osteopenia induced by pHPT.

Disclosures: ***H. Kaji****, None.*

SA485

See Friday Plenary number F485.

SA486

Normocalcemic Primary Hyperparathyroidism: Update on a New Clinical Phenotype. H. Lowe*, D. J. McMahon, M. D. Walker, M. Rubin, J. P. Bilezikian, S. J. Silverberg. College of Physicians & Surgeons, Columbia University, New York, NY, USA.

Patients with elevated parathyroid hormone (PTH) and consistently normal serum calcium levels in whom secondary causes of high PTH have been excluded may represent the earliest presentation of primary hyperparathyroidism (PHPT). We update data on 37 patients (mean age 59 yrs, range 32-78; 95% female) with normal corrected serum calcium (9.6±0.1mg/dl; nl: 8.6-10.4) and elevated PTH (93±5 pg/ml; nl:10-65) levels. All patients had 25(OH)vitamin D levels >20ng/dl, 24 hr urinary calcium (Uca) <350 mg/d, normal renal, hepatic, and thyroid function, and no malabsorption. Classical features of PHPT included a history of kidney stones in 5 (14%), and fragility fractures in 4 (11%). In 11 subjects (30%), 1,25$(OH)_2$ D levels were elevated, while 7 (18%) had high Uca. BMD did not show evidence of preferential bone loss at the more cortical distal 1/3 radius (LS T-Score: -2.03±0.25; FN T: -1.84±0.18; RAD T: -1.74±0.22). Osteoporosis (OP; found in 59%, n=22 patients) was more common at the LS (38%) and FN (41%) than at the RAD (22%).

Patients were followed for 4±0.3 yrs (1-9 yrs), during which 8 subjects (22%) became frankly hypercalcemic. Those who developed hypercalcemia were older (64±2y vs. 57±2, P=0.02) and had higher baseline serum calcium levels (9.9±0.2 mg/dl vs. 9.5±0.1, P<0.001). Seven patients (5 normocalcemic) developed NIH surgical criteria for hypercalcemic PHPT: marked hypercalciuria in 3; recurrent kidney stone in 1; traumatic fracture in 1; new OP in 4. Repeat BMD testing (n=33) demonstrated that 51% lost ≥5% and 33% lost ≥10% BMD at ≥1 skeletal sites, with similar declines seen at all sites. Osteoporosis at baseline did not predict who would lose ≥10% BMD (with OP: 7/22, 32%; no OP: 4/15, 27%). Significant bone loss, new OP or new kidney stone developed in 15/37 (40%).

Sestamibi scans were positive in 13/21 cases. Ten patients had successful PTX: 5 hypercalcemic and 5 normocalcemic patients with NIH surgical criteria: kidney stones (n=1), OP (n=4). Pathology in normocalcemic patients showed adenoma (n=2) and hyperplasia (n=3), while hypercalcemic patients had single adenoma (n=3), double adenoma (n=1) or hyperplasia (n=1). One yr post-PTX BMD rose 5% at LS, but was unchanged at FN and RAD.

In summary, these patients with normocalcemic PHPT do not have the typical profile of hypercalcemic PHPT. They present with more signs and symptoms, and develop more complications over time. Despite this, >75% of patients remain normocalcemic. There are no predictors of the timing or onset of hypercalcemia. We conclude that these patients do not represent emerging asymptomatic PHPT. Instead they may be an early presentation of a more symptomatic variant of PHPT, some of whom may never become hypercalcemic.

***Disclosures:* *H. Lowe**, None.*
This study received funding from: NIH.

SA487

Antioxydants (Phytoestrogens and Quercetin) Are Potentials Agents Against Rheumatoid Arthritis. I. Henry-Desailly*[1], E. Flipon*[2], P. Lefauveau*[1], D. Ursu*[1], C. Benneteau-Pelissero*[3], S. Kamel*[4], M. Brazier*[4], P. Fardellone*[2]. [1]Rheumatology, University Hospital, Amiens, France, [2]Rheumatology - Inserm Eri12, University Hospital, Amiens, France, [3]ENITA, Bordeaux, France, [4]Inserm eri12, Faculty of Pharmacy, Amiens, France.

Objective: To estimate the influence of alimentary phytoestrogens and quercetin alimentary intake on rheumatoid arthritis (RA).

Methods:: We performed a case control study comparing 30 women having Rheumatoid Arthritis with 30 controls women matched on BMI and age, (between 45 to 65). The flavonoids exposure was assessed by biological dosages in plasma (pl) and urines (u) by an ELISA immuno-assay (genistein, dadzein, equol) and by liquid chromatography-masss prectrometry (quercetin).

Some patients were excluded if under medical condition (like pregnancy or hormonal dependent cancers,) or treatments (oral contraceptive, hormonal replacement for menopause) which could influence their hormonal status.

We compared the means of phytoestrogens concentration in plasma and urines of the two groups, using matched controls "t" test after log-transformation.

Results:: Phytoestrogens and quercetin concentrations according to the two groups are shown in the table.

Phytoestrogens and Quercetine (ng/ml)	RA Mean (SD)	Controls Mean (SD)	p
Genistein (u)	225.0 (582,0)	178 (501,4)	0.135
Genistein (pl)	20.9 (83.8)	70,3 (262,3)	0.778
Daidzein (u)	677.7 (1619.7)	359 (786,6)	0.144
Daidzein (pl)	16,4 (57.6)	31.5 (112,2)	0.752
Equol cor (u)	10.7 (33.8)	48,2 (108,4)	**0.029**
Equol cor (pl)	0.83 (3.7)	0.87 (3,4)	0.991
Quercetine (u)	19,02 (2,05)	18,2 (2,9)	0.530
Quercetine (p)	7,2 (3.2)	10,8 (4,2)	**0.038**

Equol in urines and quercetin in plasma are he only flavonoids for which we observed a significant difference between the two groups Equol u (p= 0.029) and Quercetin pl (p=0.038).

Equol is chemically unique among the isoflavone, it is the major metabolite of the phytoestrogen daidzein. This phytoestrogen has one of the strongest estrogen activity and could interact with the immunologic system.

Quercetin, flavonal widely distributed in plants has antioxydant properties with antiproliferative, anti-inflammatory and immunosuppressive activities.

Conclusion: Our results suggest that equol and quercetin have a protective effect toward the onset of Rheumatoid Arthritis, thanks to their antioxydants and anti-inflammatory properties.

***Disclosures:* *E. Flipon**, None.*
This study received funding from: Hospital university of Amiens.

SA488

See Friday Plenary number F488.

SA489

The Association Between Several Factors Affecting Aortic Calcification and Bone Mineral Density In Postmenopausal Women. W. H. Choi, H. Han*, S. M. Hong*, Y. H. Ahn*. Endocrinology, Hanyang University, Seoul, Republic of Korea.

Background: Aortic calcification increases with age and several epidemiologic studies shows that it is linked to cardiovascular mortality such as myocardial infarction and stroke. Elderly person whose degree of aortic calcification increases greater tends to lose more bone than those whose gain for aortic calcification is minimal. Osteoclastic potential is greater in preosteoclasts from bone marrow in hyperlipidemic compared with normal mice. Therefore we are to evaluate the factors that contributes to vascular calcium accumulation and the association between Lipid profile and bone mineral density(BMD)

Methods: Postmenopausal women who visited the health promotion center at Hanyang Univ. hospital from Jan 2003 to Sep 2007 have taken baseline checkup including history taking, physical examination, and blood sampling. Lumbar spinal and femoral BMD was measured by dual energy X-ray absorptiometry(DXA).The presence of aortic calcification was identified by Abdominal CT. According to the extent of sum of aortic calcification observed in all cross sectional area, all patients were divided to 3 groups: None(No calcification), Mild to moderate(0-180°), Severe(180-360°)

Results: Patients with aortic calcification had significantly lower BMD than those without aortic calcification. High triglyceride and low HDL cholesterol was significantly associated with aortic calcification. Diabetic patients had a higher incidence of aortic calcification than non-diabetic patients(83% vs 49%;P value<0.01), and they had significantly higher triglyceride and lower HDL cholesterol levels than non-diabetic patients. As the degree of aortic calcification increases, BMD especially femoral BMD tends to decreases. However, none of the lipid profiles was significantly correlated with BMD.

Conclusion: There is a reciprocal relationship between degree of aortic calcification and bone mineral density. Among other cardiovascular risk factors, DM is a potent risk factor for aortic calcification. In addition to metabolic stress induced by hyperglycemia, Dyslipidemia may contribute to vascular calcium accumulation. Although dyslipidemia including high TG and low HDL is significantly associated with aortic calcification, there is no meaningful correlation between serum lipid level and BMD.

***Disclosures:* *W.H. Choi**, None.*

SA490

Elevated Serum Ionic Fluoride Levels in Post-menopausal Women is Due to the Enhanced Release of Fluoride from Bone. K. Itai*[1], M. Ohsawa*[1], K. Tanno[1], T. Onoda*[1], T. Sato*[1], T. Kuribayashi*[1], K. Sakata*[1], A. Okayama*[2]. [1]Hygiene and Preventive Medicine, Iwate Medical University, Morioka, Japan, [2]The First Institute of Health Service, Anti-Tuberculosis Association, Tokyo, Japan.

Serum ionic fluoride (SIF) levels may reflect changes in bone metabolism along with osteoporosis, especially in the middle-aged and elderly post-menopausal women. However, useful references of age-specific SIF levels and the confounding factors significantly associated with SIF levels have not been fully elucidated in a general population. The purpose of this study is to determine sex- and age-specific mean levels of SIF and to estimate confounding factors associated with SIF levels. A total of 332 participants (167 men and 165 women) were enrolled. Fasting blood samples were collected from all participants. SIF levels were measured by the flow injection method with an ion selective electrode as a detector. A multiple regression analysis was performed to determine confounding factors associated with SIF levels. Participants were divided into sex- and age-specific groups and multivariate-adjusted SIF levels were compared between the groups using ANCOVA after adjustment for the associated factors separately by sex. The crude means of SIF levels (μmol/l) were 0.495 in men and 0.457 in women, respectively. Crude SIF levels were positively associated with age in both sexes (see table). Estimated glomerular filtration rate (eGFR) and plasma glucose (FPG) levels were independently associated with SIF levels in both sexes. Menopause status was independently associated with SIF levels in women. Crude and adjusted SIF levels in post-menopausal women were significantly higher than those in pre-menopausal women. Decreased glomerular filtration rate was the most powerful factor that increases SIF levels in men. Elevated SIF levels in post-menopausal women indicate that the enhanced fluoride release from bone exists accompanied with the accelerated bone resorption after menopause.

Crude and adjusted serum ionic fluoride levels stratified by sex and age classes

Age classes (years)	40-49	50-59	60-69	trend P
Male subjects (n)	55	53	59	-
Crude, μmol/l	0.454 ± 0.158	0.509 ± 0.159	0.519 ± 0.185	0.045
Adjusted (95% CI), μmol/l	0.463 (0.420-0.506)	0.513 (0.469-0.557)	0.508 (0.466-0.550)	0.143
Female subjects (n)	53	57	55	-
Crude, μmol/l	0.383 ± 0.135	0.470 ± 0.166	0.516 ± 0.151	<0.001
Adjusted (95%CI), μmol/l	0.439 (0.388-0.489)	0.461 (0.423-0.500)	0.471 (0.429-0.514)	0.418

***Disclosures:** **K. Itai**, None.*

SA491

See Friday Plenary number F491.

SA492

Improved Forearm Bone Mineral Density after Treatment with Vitamin D_3 in a Patient 30 Years after Bariatric Surgery. S. E. Williams[1], A. A. Licata[2]. [1]Center for Nutrition and Metabolic Medicine, Greene Memorial Hospital, GMH Health Center, Xenia, OH, USA, [2]Metabolic Bone Center, Cleveland Clinic Foundation, Cleveland, OH, USA.

Our purpose is to report a case of severe secondary metabolic bone disease in which a statistically significant increase in forearm bone mineral density occurred as a result of vitamin D_3 supplementation.
A 76-year-old nonambulatory woman who underwent jejuno-ileal bypass (JIB) bariatric surgery in 1974 presented to our metabolic bone center with hypocalcemia, hypovitaminosis D, hyperparathyroidism, proximal weakness, and debilitating bone pain. The DXA T-scores for total hip and forearm were -5.0 and -6.5 standard deviations, respectively. Oral cholecalciferol and calcium were prescribed, and dose adjustments were made in an effort to promote adequate absorption in response to periodic laboratory tests. Our patient required 100,000 IU cholecalciferol and 1800 mg calcium citrate on a daily basis due to iatrogenic malabsorption.
The 25-hydroxyvitamin D level, undetectable at first presentation, returned to normal limits during an 8-month period of aggressive oral supplementation. Lab data are noted in Table 1. During this period the distal forearm bone mineral density increased by 17.9%; there was no significant change in the left total hip.

Table 1
Data Before and After Supplementation with Cholecalciferol[1]

Serum Factor	Ref Range	Baseline	After
Albumin (g/dL)	3.4 - 4.7	2.8	3.8
Creatinine (mg/dL)	0.7 - 1.4	1.2	1.4
Total Calcium (mg/dL)	8.5 - 10.5	7.4	8.7
Alkaline Phosphatase (U/L)	40 - 150	456	333
Parathyroid Hormone (pg/mL)	10 - 60	488	190
25-hydroxyvitamin D (ng/mL)	31 - 80	< 7.0	37

[1] First three months: 50,000 IU cholecalciferol twice weekly, patient stopped calcium supplement due to dyspepsia; 4th month: 600 mg calcium carbonate tid with meals plus 50,000 IU cholecalciferol daily; 5th month: increased cholecalciferol to 100,000 IU daily, and changed to calcium citrate.

We conclude that supplementation with vitamin D_3 and calcium alone can improve forearm bone density in certain skeletal problems and that clinicians must carefully interpret abnormal DXA data, since it may not always reflect primary osteoporosis.

***Disclosures:** **S.E. Williams**, None.*

SA493

(22-52) Adrenomedullin Prevents Systemic Bone Loss Related to Inflammation in Mice. C. Asensio*[1], M. Ah-Kioon*[1], H. Ea*[1], B. Uzan*[1], A. Kadri*[1], C. Bazille*[1], C. Marty*[1], C. Collet*[1], J. Launay*[2], F. Liote*[1], M. E. Cohen-Solal[1]. [1]Hôpital Lariboisière, INSERM U606, Paris, France, [2]Hôpital Lariboisière, Laboratoire de Biochimie, Paris, France.

Rheumatoid arthritis is associated with focal and systemic bone loss involving many inflammatory factors. Among them, adrenomedullin (ADM) reduces inflammation in collagen-induced arthritis (CIA) mice model. Moreover, ADM exerts *in vivo* anabolic effects on bone along with *in vitro* anti-apoptotic effects on osteoblast cells. (22-52)ADM, a fragment peptide which binds to ADM receptor, might act as an antagonist of ADM *in vitro* but its *in vivo* effects are unknown. We evaluated the action of ADM and (22-52)ADM, on systemic bone loss in a CIA mice model. DBA/1 mice were immunized with bovine type II collagen and treated with ADM or (22-52)ADM (1.2 μg/g for both) or saline. Total body and vertebral bone mineral densities (BMD) were measured at baseline and at sacrifice (D45), and expressed as Delta BMD. Arthritic scores were evaluated in a blinded manner and histological studies of joints and bone were performed at sacrifice. Histomorphometric parameters were evaluated at the lumbar vertebrae. Bone resorption was quantified using the urinary deoxypyridinoline assay. At 45 days after disease onset, ADM and (22-52)ADM decreased arthritic score compared to saline (6.8±1.6 and 5.8±2 vs 11±3, respectively).Moreover, total histological score (inflammation, pannus and cartilage) was decreased with ADM compared to controls (1.30±0.07 vs 3.78±0.26) and further decreased with (22-52)ADM (0.56±0.04 vs 3.78±0.26, p<0.05). Furthermore, CIA induced systemic bone loss, as evidenced by Delta BMD, compared to the naive group (total body (TB): 17.9±2.7% vs 25.5±0.2%; vertebrae (V): 16.8±2.8% vs 29.4±7.7%). Compared to CIA mice treated with saline, ADM partly prevented CIA-induced bone loss (23.6±2.3% vs 17.9±2.7% at TB and 20.0±2.8% vs 16.8±2.8% at V, p = NS). Moreover, (22-52)ADM also increased BMD (25.7±2.6% vs 17.9±2.7% at TB and 33.2±3.6% vs 16.8±2.8% at V, p<0.05). Indeed, (22-52)ADM increased trabecular bone volume (12±0.6 vs 10±0.5%, p<0.05) as well as trabecular thickness (28.3±0.8 vs 25.2±0.8 μm, p<0.05) suggesting an effect on bone formation. Urinary deoxypyridinoline levels were increased in CIA compared to naives (14.09±0.92 vs 8.22±0.47, p<0.05) but were unchanged with ADM and (22-52)ADM. In conclusion, both ADM and (22-52)ADM prevented bone loss in CIA mice. (22-52)ADM could prevent bone loss by maintaining osteoblast activity. Although the mechanism of action of each peptide remains to be determined, these molecules might represent a potential interest for the prevention of inflammatory-related bone loss.

***Disclosures:** **C. Asensio**, None.*
This study received funding from: Rhumatisme et Travail.

SA494

See Friday Plenary number F494.

SA495

Withdrawn

SA496

Vitamin D Status and Bone Remodeling Marker CTx-I in Patients with Early Rheumatoid Arthritis (RA): Association with Disease Activity and Joint Damage. M. L. Gonzalez-Casaús*[1], P. Aguado*[2], M. C. Ordoñez*[2], A. Cabezon*[3], D. Pascual-Salcedo*[3], A. Balsa*[2], E. Martin-Mola*[2]. [1]Biochemistry, Hospital Central de la Defensa, Madrid, Spain, [2]Rheumatology, Hospital Universitario La Paz, Madrid, Spain, [3]Immunology, Hospital Universitario La Paz, Madrid, Spain.

Background: Vitamin D (VD) is a potent regulator of calcium homeostasis and musculoesqueletal function and may also have immunomodulatory effects. The influence of VD on RA is not well defined. The type I collagen C-telopeptide bone breakdown product (CTX-I) has been related with joint damage in chronic persistent arthritis
Objectives: To study the association of baseline 25 (OH) D3 serum level with disease activity and with functional scores in patients with early RA, and to investigate the relationship between baseline CTX-I and radiographic joint damage
Methods: Patients attending an early arthritis clinic and showing signs of probable RA were studied. Disease activity was recorded at initial evaluation and every 6 months thereafter, taking into account pain, patient and physician assessment, articular indices, acute phase reactants and functional capacity (HAQ). In addition, rheumatoid factor (RF) and cyclic citrulinated peptide antibody levels (anti-CCP) were determined. X-Rays of hands and feet were taken at initial evaluation and every 12 months. Basal levels of serum

calcidiol (SC) and CTX-I were determined by electrochemiluminiscence (ECLIA) using an Elecsys analyser (Roche). X-Rays were evaluated by the Sharp-van der Heijde method, following a chronological order and by pairs. Patients were treated according to physician´s criteria and biological agents were not used. Results were compared by t test, ANOVA (Schfee), and correlation studies (Pearson and Spearman), as required

Results: 165 patients with RA were studied; 75.8% were female, mean age was 53 ±17 years and disease duration, 16 ± 10 weeks. 161 (97%) patients fulfilled ACR criteria for RA during follow-up. At baseline, mean SC was 25.6 ±10.7. SC levels were not related to RF or anti-CCP antibodies. Eighty-two percent of patients had a vitamin D insufficiency (<30 ng/ml). Basal SC was inversely correlated with patient global disease assessment (r = -0.4, p=0.05), and with the number of painful joints, both at entry and during the first 2 years (r = -0.23, p=0.04). Basal CTX-I levels were correlated with radiological damage at all time points; at entry (r=0.46; p= 0.006), at one year (r=0.41; p=0.03) and at 2 years (r=0.38, p=0.05)

Disclosures: ***M.L. Gonzalez-Casaús****, None.*

SA497

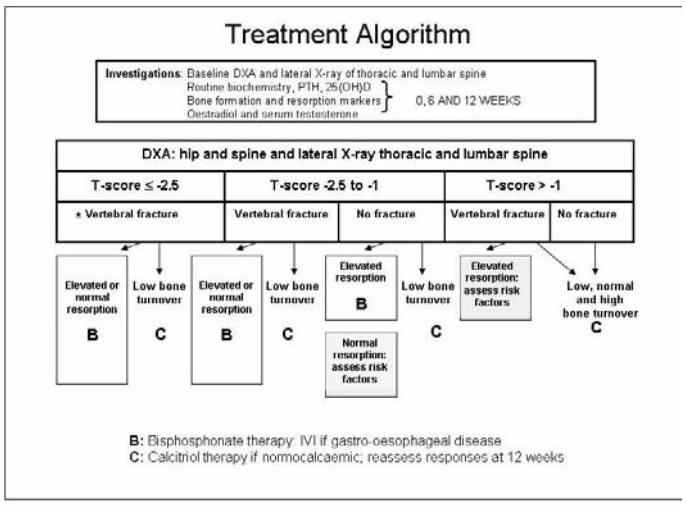
Application of a Treatment Algorithm to Avoid BMD Loss and Limit Bisphosphonate Use after Kidney and Kidney Pancreas Transplantation. R. Mainra*[1], G. J. Elder[2]. [1]Division of Nephrology, St. Paul's Hospital, Saskatoon, SK, Canada, [2]Centre for Transplant and Renal Research, Westmead Millenium Institute, Sydney, Australia.

Despite reduction of glucocorticoid exposure in current immunosuppressive regimens, BMD levels often fall after kidney (K) and kidney pancreas (KP) transplantation and the incidence of post-transplant fractures is high. In meta-analysis, bisphosphonates and vitamin D reduce loss of BMD at the spine and hip but both have potential adverse effects. We studied the consequences of allocating patients to bisphosphonate and calcitriol therapy using a treatment algorithm.

In this prospective study, 155 transplant recipients (K: 92 and KP: 63) were followed for 12 months. Kidney recipients differed from KP recipients by age (48±12 vs. 38±7 years, p<0.0001), dialysis vintage (37±5 vs. 17±2 months; p<0.01) and haemo. vs. peritoneal dialysis (65% vs. 44%, p<0.01). Transplant recipients were assessed with bone turnover markers (serum osteocalcin and urinary deoxypyridinoline/creatinine), levels of iPTH, vitamin D and sex hormones 4-6 weeks after transplant. BMD by DXA and lateral spine X-ray were performed. Patients were allocated to bisphosphonate or calcitriol according to an algorithm based on these results and assessment of standard risk factors. Patients with 25OHD levels <60 nmol/L received cholecalciferol.

Using the algorithm, 56% of patients received bisphosphonates. Age was similar between treatment groups as was gender and transplant type. More patients allocated to bisphosphonates were on haemodialysis pre-transplant (66% vs. 43%; p=0.02) and were of longer dialysis vintage (32±4 vs. 22±6 months; p=0.006). Patients receiving bisphosphonates had lower baseline BMD at the lumbar spine and femoral neck (p<0.0001 for each). At 12 months, BMD increased in bisphosphonate treated patients at the lumbar spine and femoral neck (p=0.0012 and p=0.002 respectively). BMD in non-bisphosphonate treated patients did not change significantly at any site.

When patients with chronic kidney disease are transplanted, post-transplant laboratory and BMD data may be difficult to interpret due to pre-existing renal osteodystrophy. Targeting therapy using this algorithm resulted in reduced bisphosphonate exposure and potential drug-related risks, while maintaining or improving BMD at all sites.



Disclosures: ***R. Mainra****, None.*

SA498

Change of Pathological Bone Lesion in Recipients with Renal Osteopathy after Renal Transplantation. T. Tokumoto*[1], T. Nozaki*[1], K. Yoshida*[1], H. Suzuki*[1], T. Akiba[2], K. Tanabe*[2]. [1]Kidney Center, Dept. of Urology, Toda Central General Hospital, Saitama, Japan, [2]Kidney Center, Tokyo Women's Medical University, Tokyo, Japan.

OBJECTIVES; We expect if chronic renal failure (CRF) is improved after renal transplantation (RTx), dialysis osteopathy is also recover to normal in bone lesion. Nevertheless, it is controversial whether bone lesion is really improved after RTx. In this study, we evaluated whether dialysis osteopathy was pathologically improved after RTx.

MATERIALS AND METHODS; Forty-one cases that underwent living related RTx at Toda central general hospital from January, 2004 were enrolled in this 28 males and 13 females. The periods of hemodialysis (HD) were an average of 40.2 months. The immunosuppression was basically triple drug therapy such as FK, MMF and Steroid. The parameter of Ca, P, whole PTH (w-PTH) and metabolic bone marker and bone density (DXA) were examined with relation to dialysis osteopathy in before RTx, and 1 year after RTx. In addition, bone biopsy was performed after having made osteal labeling twice in principle before bone biopsy.

RESULTS; All cases are survival and the renal grafts are functioning well in all cases. The mean level of Ca and P before RTx were 9.4mg/ml and 5.5mg/dl, respectively. The mean level of w-PTH was 73.4 pg/ml before RTx. The mean level of ALP was 262.1 U/l before RTx. The mean level of BAP was 29.6 U/l before RTx. The mean of bone volume (BV/TV) before RTx was 18.0%. The mean of osteoid volume (OV/TV) before RTx was 3.6%. The mean of fibrosis volume (Fb.V/TV) before RTx was 0%. The mean of bone formation rate (BFR/BV) before RTx was 25.5 %/year. Both of BV/TV and BFR/BV after RTx were increased pathologically. On the other hand, Fb.V/TV and OV/TV were also remarkably increased. Out of 41 cases, 30 cases (73.2%) were pathologically diagnosed as renal osteodystrophy, 7 cases (17.1%) were aplastic osteopathy, 2 cases (4.9%) were renal osteodystrophy or aplastic osteopathy, one case (2.4%) was osteitis fibrosa and one case (2.4%) was osteomalacia by bone biopsy at RTx. Six cases were already underwent the bone biopsy at 1 year after RTx. Out of these cases, 3 cases were renal osteodystrophy, 2 cases were aplastic osteopathy, and one case was renal osteodystrophy or aplastic osteopathy at RTx. Of 6 cases, 2 cases were pathologically diagnosed as renal osteodystrophy, 2 cases were mixed type, and 2 cases were osteomalacia at 1 year after RTx.

CONCLUSIONS; The bone metabolism was greatly improved at one year after RTx, but bone lesion in recipients with renal osteopathy did not seem to be recover pathologically. In particular, the tendency strongly resisted in case of aplastic osteopathy. The further examination by metabolic bone marker and bone biopsy will be needed in future.

Disclosures: ***T. Tokumoto****, None.*

SA499

Bone Metabolism in Long-term Kidney Transplantation Recipients. A. Suzuki[1], H. Sasaki*[2], S. Yamamoto*[1], S. Sekiguchi[1], M. Shibata[1], S. Asano[1], K. Hoshinaga*[2], M. Itoh*[1]. [1]Division of Endocrinology, Department of Internal Medicine, Fujita Health University, Aichi, Japan, [2]Department of Urology, Fujita Health University, Aichi, Japan.

Recent pharmacological advances in immunosuppressive therapy and transplant techniques have been improving long-term survival of graft recipients. Post-transplantation bone diseases negatively affect quality of life of solid organ recipients, because osteoporosis is prevalent in more than half of them. Bone loss during the first year after transplantation is most pronounced, but bone density development in long-term transplant recipients is still controversial. In the present study, we conducted cross-sectional study for bone metabolism in long-term survivors after kidney transplantation. Subjects were post-renal transplantation recipients (n=31, M/F=15/16, age 54.8 ± 8.9). Mean duration of hemodialysis was 116 ± 83.7 months. Mean duration after renal transplantation was 14 ± 3 years (9-20 years). All the patients were prescribed methylprednisolone (4.07 ± 0.84 mg/day) with various immunosuppressive agents (ciclosporin, 28/31; mizoribine, 13/31; tacrolimus hydrate, 3/31; mycophenolate mofetil, 5/31). Mean concentrations of serum corrected calcium, phosphate and creatinine were 9.8 ± 0.6 mg/dl, 2.9 ± 0.4 mg/dl and 1.19 ± 0.46 mg/dl, respectively. Mean serum 25-hydroxyvitamin D level was 20.3 ± 4.3 ng/ml, while serum 1,25-dihydroxyvitamin D3 was 48.5 ± 15.7 pg/ml. Mean concentration of intact parathyroid hormone (iPTH) (142.8 ± 74.3 pg/ml) was about three times higher than that of whole parathyroid hormone (wPTH)(54.3 ± 34.3 pg/ml). Serum iPTH and wPTH levels were negatively associated with the duration after kidney transplantation, but not with the duration for hemodialysis. In summary, these findings suggest us that long-term survivors after kidney transplantation have less persistent hyperparathyroidism, and that there is huge difference between serum iPTH and wPTH concentrations in post-kidney transplantation recipients.

Disclosures: ***A. Suzuki****, None.*

SA500

See Friday Plenary number F500.

SA501

Marked Vitamin D Deficiency in Recent Heart and Liver Transplant Recipients. E. M. Stein[1], A. Cohen[2], M. Freeby*[2], S. Kokolus*[2], D. Mancini*[2], S. Restaino*[2], R. Brown*[2], D. J. McMahon[2], E. Shane[2]. [1]Columbia University, New York, NY, USA, [2]Medicine, Columbia University College of Physicians and Surgeons, New York, NY, USA.

Vitamin D deficiency is an increasingly recognized condition with many serious sequelae. Several factors place patients with end-stage organ failure at particular risk for vitamin D deficiency, including limited sunlight exposure and hepatic dysfunction, resulting from intrinsic liver disease or in heart failure patients, hepatic congestion.
Subjects were recruited as part of a randomized trial comparing two bisphosphonates for prevention of bone loss after transplantation. Serum 25-hydroxy vitamin D (25OHD: DiaSorin Chemiluminescent assay) was measured immediately after heart or liver transplantation, and before bisphosphonate therapy.
Of 65 subjects studied (mean age: 53 ±11 years), 44 (68%) received heart and 21 (32%) received liver transplants. Subjects were ethnically diverse, 57% Caucasian, 14% African American, 20% Hispanic, 2% Asian and 8% of other ethnicity. Vitamin D levels did not differ by ethnicity. The distribution of serum 25OHD is shown in the table:

Serum 25OHD (ng/ml)	Study Population (n=65)	Heart Transplant (n=44)	Liver Transplant (n=21)
Mean ± SD	17.1 ± 9.3	19.2 ± 9.4	12.7 ±7.2
<10	12 (18%)	4 (9%)	8 (38%)
10-<20	36 (55%)	26 (59%)	10 (48%)
20-<30	12 (18%)	10 (23%)	2 (10%)
≥30	5 (8%)	4 (9%)	1 (5%)

Low 25OHD levels were nearly ubiquitous; 92% had levels below 30 ng/ml, the threshold commonly used to denote sufficiency. Six had levels below 7 ng/ml, the lower limit of detection of the 25OHD assay. The distribution of vitamin D levels was significantly lower among liver than heart transplant recipients (p=0.01). Severe vitamin D deficiency was particularly widespread in liver transplant recipients; 8 (38%) had levels <10 ng/ml and of these, 5 (24%) had undetectable levels.
Severe vitamin D deficiency is extremely prevalent among heart and liver transplant recipients. Patients with end-stage liver disease are at exceptionally high risk, likely because of disease related factors such as malabsorption, and impaired hepatic 25-hydroxylation of vitamin D. Patients with severe vitamin D deficiency are at risk for hypocalcemia and tetany after treatment with intravenous bisphosphonates. It is imperative to assess vitamin D status and correct vitamin D deficiency in all patients presenting for transplantation, particularly if intravenous bisphosphonates are prescribed to prevent transplant-related bone loss.

***Disclosures: E.M. Stein**, None.*
This study received funding from: Novartis.

SA502

A Retrospective Study Evaluating the Relationship of Vitamin D 25 OH and Free Testosterone Levels with Changes in Hip Bone Density after Liver Transplantation. R. K. Bhattacharya[1], M. Hagan*[1], R. Krebill*[2], B. Lukert[1], L. Graves[1]. [1]Endocrinology, University of Kansas, Kansas City, KS, USA, [2]Biostatistics, University of Kansas, Kansas City, KS, USA.

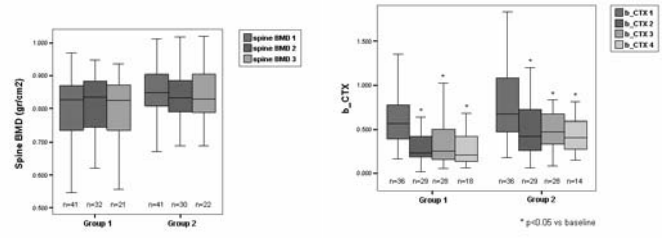
Osteoporosis and increased fracture risk are common complications following orthotropic liver transplantation (OLT). Pre-transplant markers have not been clearly identified to predict post-transplant changes in bone density.
Aims of this study: 1. To evaluate changes in BMD post transplantation and to correlate these changes with baseline Vitamin D 25 OH and free testosterone (in males) levels; 2. To explore the relationship between BMD changes and free testosterone levels pre and post transplantation.
Methods: This retrospective study evaluated the baseline characteristics of 32 individuals (17 men, 15 women) prior to liver transplantation. The individuals where followed for an average of 425 days post-transplantation and the BMD of the spine and hip, free testosterone (men only) and Vitamin D 25 OH were measured. A paired t-test was used to contrast pre -post changes in BMDs after liver transplantation. Spearman's correlation was used to determine the correlations between several parameters
Results: There was a significant decrease in hip BMD after transplantation (4.29%, p<.001). Pre-transplant free testosterone positively correlated with percent change in hip BMD in males (r =.5827, p=.028). Despite having only 12 males with testosterone measurements done post-transplantation, there was a positive correlation with hip BMD (r =.77273, p=.0053). Pre-transplant free testosterone also correlated positively with post-transplant free testosterone (r=.7135, p=.0092) .
In the entire population the study found no significant change in spine BMD after transplantation. The pre-transplant and post-transplant levels of Vitamin D 25 OH had a significant correlation on the percent change of spine BMD after transplantation (pre-transplant levels: r =0.5495, p=.003, post-transplant levels: r =.559, p=0.0104).
Conclusion: This study is consistent with other studies demonstrating bone loss following organ transplantation in the first year. Our findings revealed a correlation between 25-OH D and changes in bone density following liver transplantation. In men, free testosterone also correlated with the change in bone density of the hip after transplantation. This study is limited by the small patient numbers. Further studies are needed to confirm these results and to evaluate a true cause and effect. Future studies should be undertaken to evaluate the potential effect of correcting vitamin D and testosterone deficiency to affect BMD changes.

***Disclosures: R.K. Bhattacharya**, Solvay 3.*
This study received funding from: Solvay.

SA503

Effect of Risedronate in Liver Transplantation Patients with Low Bone Mass. S. Guadalix*, G. Martinez, B. Cobaleda*, C. Vargas*, D. Lora*, E. Jódar, J. Meneu*, E. Moreno*, F. Hawkins. University Hospital 12 de Octubre, Madrid, Spain.

Osteoporosis is found in up to 50% of transplant recipients, but the most appropriate preventive measures are not clearly established. The purpose of this study is to analyze the preventive effect of oral risedronate on bone loss in patients with low bone mass detected after liver transplantation (LTx).
Patients and Methods: This is a randomized open-label single center prospective study. Eighty-three LTx patients (64 males, 19 females; age 54.8 ± 10.6 years) with lumbar and/or femoral T-score <-1 have been included. After transplantation (mean time 34±21 days) patients were randomly assigned to one of two treatment arms: Group 1 (n=41) received oral risedronate (35 mg once weekly) plus calcium (1000 mg/day) and vitamin D (800 IU); Group 2 (n=42) received calcium and vitamin D at same doses. Primary endpoint was bone mineral density (BMD) change at lumbar spine (L1-L4) and femoral regions (measured at baseline, 6 and 12 months after LTx with an Hologic QDR 4500w densitometer); secondary endpoints include changes in serum β-CTX and P1NP. Results: No differences were found in baseline characteristics between groups. Forty-three patients (21/22) have completed 12 months of treatment so far. No significant changes in BMD were observed in any group, neither at spine or hip. In Group 1, serum β-CTX decreased after 3 months (p=0.008), 6 months (p<0.05) and 12 months (p=0.008). In Group 2, a significant decrease in β-CTX was also observed. Serum P1NP increased in Group 2 after 3, 6 and 12 months of treatment (p<0.05). At baseline, 90% of patients had 25-OH vitamin D_3 below 30 ng/ml. In both groups 25-OH vitamin D_3 levels increased at three months (p<0.001), maintaining this level throughout the study. Treatment was well tolerated in both groups. Conclusions: Preliminary data of our study suggest: 1) risedronate effectively decreases bone resorption after LTx , and 2) LTx patients have low baseline vitamin D levels.



***Disclosures: G. Martinez**, None.*
This study received funding from: Fundación Mutua Madrileña (project number 2005/072).

SA504

12 Months of Teriparatide Therapy Increases Ultradistal Radius Bone Strength in Severely Osteoporotic Postmenopausal Women. H. M. Macdonald[1], D. A. Hanley*[2], S. K. Boyd[1]. [1]Mechanical & Manufacturing Engineering, University of Calgary, Calgary, AB, Canada, [2]Medicine, University of Calgary, Calgary, AB, Canada.

Teriparatide, the amino terminal fragment of parathyroid hormone [PTH (1-34), Forteo™], is an established anabolic therapy for osteoporosis treatment. To date, one study has used non-invasive high-resolution pQCT (HR-pQCT) to quantify changes in bone microarchitecture following PTH administration (JBMR 2007 22:S24). However, PTH-mediated changes in estimated bone strength have not been investigated using novel, subject-specific finite element (FE) analysis with HR-pQCT scans. Thus, we aimed to determine the effects of PTH-therapy on estimated bone strength (ultimate failure load) at the ultradistal (UD) radius in severely osteoporotic postmenopausal women. We used HR-pQCT (Scanco Medical, Switzerland) and FE analysis (Bone 2007 41:129-37) to evaluate changes in estimated UD radius bone strength in 16 women (mean age 66 ± 14 yrs) diagnosed as severely osteoporotic (BMD T-score < -3.5 SD). All women had received bisphosphonate therapy prior to starting on PTH (1-34). We acquired HR-pQCT scans at baseline and 6- and 12-months after initiation of 20 ug/day of PTH (1-34). We analyzed 6-month data for all subjects and 12-month data for 7 of 16 subjects (remaining scans to be analyzed by August 2008). In addition to FE analysis we performed a standard microarchitectural morphological analysis. We used the Wilcoxon Signed Ranks test to compare outcomes before and after treatment. None of the women sustained a fracture during the study. After 6 months, we observed a *decrease* in bone strength (-5.9%, Table) despite a small increase in trabecular bone volume/total volume (BV/TV). Six months of PTH therapy was also associated with a decrease in cortical density (Dcort) and thickness (C.Th). In contrast, after 12 months we observed a trend for a small *increase* (+4.3%) in bone strength together with trends for increases in BV/TV, trabecular number (TbN) and thickness (Tb.Th) and continued decreases in Dcort and C.Th. These preliminary data suggest that 12 months of teriparatide therapy may increase UD radius bone strength in severely osteoporotic women, primarily through positive effects on cancellous bone morphology. Further study is needed to determine the influence of the apparent treatment-related increase in cortical porosity (evidenced by the decrease in Dcort) on radius bone strength.

6- and 12-month Percent Change in HR-pQCT outcomes				
Variable	6-Month %Change Median [Interquartile Range (IQR)] (n=16)	*p*-value	12-month %Change [Median (IQR)] (n=7)	*p*-value
Estimated Failure Load	-5.9 (-7.9 - (-0.2))	0.02	4.3 (0.5 - 10.4)	0.2
BV/TV	1.9 (0.35 - 4)	0.03	3.8 (-2.3 - 6.7)	0.3
TbN	2.4 (-3.7 - 4.6)	0.4	4.3 (-7.2 - 7)	0.9
Tb.Th	1.5 (-2.8 - 8)	0.4	2.2 (-4.4 - 5.3)	0.9
Dcort	-9.2 (-11.7 - (-1.5))	0.02	-2.2 (-2.8 - 0.2)	0.2
C.Th	-1.7 (-5.2 - 0.8)	0.2	-2.9 (-7.5 - 1)	0.3

Disclosures: ***H.M. Macdonald****, None.*

SA505

Effect of Risedronate on Bone Strength and Work to Failure Determined by Finite Element Analysis and Simulation of Clinically-Measured Bone Loss and Mineralization Changes. H. Hong*, G. J. Gross*, T. E. Dufresne*, P. A. Chmielewski*, R. J. Phipps, B. Borah. Procter & Gamble Pharmaceuticals, Mason, OH, USA.

The reduction in relative fracture risk by risedronate is largely due to the reduction of bone remodeling resulting in the prevention of bone loss, preservation of architecture and increase in mineralization. These factors contribute to the relative difference in bone strength in patients with and without treatment. We performed bone loss simulations and FEA on iliac biopsies to assess bone strength in patients treated with risedronate or placebo. Changes in bone volume and mineralization were simulated to match 3 year results as measured originally by 3D micro-CT (Bone, 2004, 34:736-46; Bone, 2005, 37:1-9). We used 12 baseline biopsy images (4 placebo; 8 risedronate) with varying trabecular bone volumes (11-29%). A uniform volume (3.5 mm^3, 34 microns resolution) was used to normalize for structural variability in the biopsies. A custom program simulated bone loss through random surface erosion of bone using a 3D Euclidian distance map. The 3D images before and after simulation were directly converted into finite element models for analysis of mechanical properties by compression loading using a non-linear ABAQUS analysis. Simulation of a 26% bone volume loss to match the 3 year placebo-treated group produced deterioration of trabecular architecture that closely matched clinical results. It resulted in ~65% reduction in the apparent properties (stiffness, strength and work to failure) compared to baseline. Reducing bone volume by 26% also reduces mineral density by 26%. Reducing density alone by 26% by simulating loss of tissue mineralization (at constant bone volume) reduced strength by only ~25%, suggesting that bone architecture is a more important determinant of bone strength. To simulate the effect of risedronate treatment, mineralization levels were increased 5% to match the change observed in clinical samples. This resulted in increases in stiffness and strength of 3-4%, and no significant change in work to failure Thus, simulation of the risedronate and placebo treatment effects on trabecular bone resulted in bone strength ~70% higher for risedronate compared to placebo. In conclusion, the preservation of bone architecture is significantly more important than the increase in mineralization for preserving bone strength with risedronate treatment.

Parameter	**% Change vs baseline after 26% reduction in volume Mean (SD)**	**% Change vs baseline after 26% reduction in density Mean (SD)**	**% Change vs baseline after 5% increase in density Mean (SD)**
Stiffness	-64.9 (8.2)	-25.8 (5.8)	3.8 (1.6)
Strength	-66.5 (6.7)	-25.4 (0.3)	3.5 (0.3)
Work to Failure	-65.2 (2.3)	-26.4 (0.9)	1.7 (1.6)

Disclosures: ***H. Hong****, Procter & Gamble 5.*

SA506

Oral Strontium Ranelate Treatment Markedly Improves Implant Osseointegration. L. Maimoun*, R. Rizzoli*, P. Ammann*. Department of Rehabilitation and Geriatrics, University Hospital, Geneva, Switzerland.

The employment of metallic implantation into bone is frequently used in orthopaedics and dentistry all the more with the aging population. The process of implant osseointegration begins with a phase of a phase of bone resorption around the implant, with bleeding and inflammation, and is followed by a phase of bone formation. However, the incidence of implant failure is high due to alteration of bone turnover and tissue quality. Strontium ranelate (SR), efficacious in the treatment of osteoporosis, was shown to inhibit bone resorption, to increase bone formation and to improve bone material quality. Whether SR treatment improves implant osseointegration is not known. We measured the resistance to pull-out of 1 mm diameter titanium rods implanted into the proximal tibia of 6 month-old female rats. The implants, sandblasted and acid-etched along the surface of non threaded part, were inserted into both tibias and the rats received SR (625 mg/kg, 5/7 days, n=15), alendronate (ALN) (18 microg/kg/d, 2/7 days, n=15, positive control) or vehicles (n=15) for 8 weeks. The tibias were then removed for microtomographic histomorphometry and resistance to pull-out was tested by recording the maximal force necessary to completely loosen the implant. Nanoindentation (intrinsic bone tissue quality in the vicinity of the implant) and histomorphometry were performed (in a subgroup of n=7). Results are expressed as means±SEM. Significance of differences were evaluated by analysis of variance (* p<0.05 vs control).

	Control	Strontium ranelate	Alendronate
Pull out Force (N)	32.52±3.79	43.54±3.03*	48.40±3.05*
Hardness (mPa) trabecular bone	624.8±21.6	721.7±27.6*	654.8±23.3
Hardness (mPa) cortical bone	837.3±24.5	918.5±30.54*	822.8±25.6

Both SR and ALN improved pull-out force compared to the controls, i.e. implant osseointegration. Whereas both treatments positively influenced microarchitecture, one of the determinants of pull-out force, intrinsic bone tissue quality was improved in rats treated with SR but not in rats treated with ALN. Furthermore, dynamic histomorphometry demonstrated tetracycline labelling along the implant in rats treated with SR, but not in rats treated with ALN, compatible with the hypothesize that SR may induce bony ingrowths into the surfaces of the implants. The dose of SR of 625 mg/kg correlates to human therapeutic exposure. In conclusion, whilst both treatments induce a similar pull-out force, only strontium ranelate improves implant osseointegration by positively altering formation of bone and the intrinsic bone material quality in the vicinity of the implant.

Disclosures: ***P. Ammann****, None.*
This study received funding from: Servier.

SA507

Hip Structural Analysis Based on DXA Data in Women with Postmenopausal Osteoporosis Receiving Once-Monthly Oral Ibandronate for 12 Months. E. M. Lewiecki*[1], H. K. Genant*[2], K. Engelke*[3], T. Fuerst*[4], M. Fries*[5], M. Enslin*[5], L. A. Fitzpatrick*[5], A. Kivitz*[6]. [1]New Mexico Clinical Research & Osteoporosis Center, Albuquerque, NM, USA, [2]University of California, San Francisco, and Synarc, Inc, San Francisco, CA, USA, [3]Synarc, Inc, Hamburg, Germany, [4]Synarc, Inc., San Francisco, CA, USA, [5]GlaxoSmithKline, King of Prussia, PA, USA, [6]Altoona Arthritis & Osteoporosis Center, Duncansville, PA, USA.

Bone quality, strength, and geometry contribute to fracture resistance. This 12-month randomized, double-blind, parallel-group, multicenter study determined effects of once-monthly oral ibandronate or placebo on bone quality and strength measured by volumetric quantitative computed tomography (QCT), finite element analysis, and dual-energy x-ray absorptiometry (DXA) in 93 postmenopausal women with BMD T-scores of ≤-2.0 to ≥-5.0. The primary endpoint was total hip volumetric BMD assessed by QCT. Hip structural analysis (HSA) parameters were a secondary endpoint. Femoral DXA was performed at baseline and Month 12. HSA assessed cross-sectional geometry of the intertrochanter region, narrow neck, and femoral shaft. All analyses were exploratory. HSA changes from baseline were evaluable in 42 of 47 ibandronate patients and 37 of 46 placebo patients. Ibandronate induced greater mean percentage increases than placebo in total hip BMD by DXA (1.7% ± 2.8% vs -0.6% ± 2.5%; treatment difference 2.0% [95% CI 0.7% to 3.3%]). Cross sectional moments of inertia improved with ibandronate versus placebo at the shaft (1.9% ± 5.1% vs -0.6% ± 4.5%; treatment difference 2.0% [95% CI -0.2% to 4.2%]) and narrow neck (4.1% ± 9.4% vs -0.7% ± 7.3%; treatment difference 4.0% [95% CI -0.04% to 8.1%]) denoting increased resistance to bending and torsional loading; corresponding intertrochanter data were 4.1% ± 7.5% vs 1.2% ± 8.9%, treatment difference 2.4% (95% CI -1.4% to 6.2%). Average cortical thickness of the narrow neck, a parameter associated with hip fracture resistance, decreased with placebo (-2.1% ± 5.4%) but was maintained with ibandronate (0.3% ± 6.2%); treatment difference 2.2% (95% CI -0.6% to 5.0%). In other regions, average cortical thickness increased with ibandronate and was maintained with placebo (shaft: 2.0% ± 5.2% vs 0.2% ± 5.1%, treatment difference 0.7% [95% CI -1.6% to 3.0%]; intertrochanter: 3.3% ± 6.0% vs 1.4% ± 5.8%, treatment difference 1.3 [95% CI, -1.4% to 4.0%]). Ibandronate treatment for 12 months was associated with trends toward improvement in hip geometry relative to placebo as measured by HSA.

Disclosures: ***E.M. Lewiecki****, New Mexico Clinical Research & Osteoporosis Center, Merck, Eli Lilly, Novartis, Sanofi-Aventis, Amgen, Pfizer, Wyeth, Roche, GSK, Procter & Gamble 3; Merck, Eli Lilly, Novartis, Procter & Gamble, Sanofi-Aventis, Roche, GSK 1; Merck, Eli Lilly, Novartis, Procter & Gamble, Sanofi-Aventis, Roche, GSK, Wyeth, Servier, Amgen, Upsher-Smith 4; Board of Directors: International Society for Clinical Densitometry 5.*
This study received funding from: Roche and GlaxoSmithKline.

SA508

See Friday Plenary number F508.

SA509

QCT and FEA Assessment of Proximal Femur Bone Quality and Strength in Women with Postmenopausal Osteoporosis Receiving Once-Monthly Oral Ibandronate for 12 Months. A. Kivitz*[1], T. M. Keaveny*[2], H. K. Genant*[3], K. Engelke*[4], T. Fuerst*[5], D. Kopperdahl*[6], M. Fries*[7], G. Dasic[7], L. A. Fitzpatrick*[7], E. M. Lewiecki*[8]. [1]Altoona Arthritis & Osteoporosis Center, Duncansville, PA, USA, [2]University of California, Berkeley, and O.N. Diagnostics, Berkeley, CA, USA, [3]University of California, San Francisco, and Synarc, Inc, San Francisco, CA, USA, [4]Synarc, Inc., Hamburg, Germany, [5]Synarc, Inc., San Francisco, CA, USA, [6]O.N. Diagnostics, LLC., Berkeley, CA, USA, [7]GlaxoSmithKline, King of Prussia, PA, USA, [8]New Mexico Clinical Research & Osteoporosis Center, Albuquerque, NM, USA.

Decreased femoral bone mineral density (BMD) and cortical thinning are associated with osteoporotic hip fracture risk. We examined effects of once-monthly oral ibandronate on measures of bone strength and quality in a 12-month randomized, double-blind, placebo-controlled study. Postmenopausal women (N=97) aged 55-80 years with BMD T-scores ≤-2.0 and ≥-5.0 received oral ibandronate (150 mg/mo) or placebo. The primary endpoint was assessment of integral (cortical plus trabecular compartments) total hip volumetric BMD by quantitative computed tomography (QCT) after 12 months' ibandronate or placebo treatment in the intent-to-treat population (n=93); secondary analyses included other proximal femur sites and finite element analysis (FEA). All analyses were exploratory. *P*-values were generated post-hoc for descriptive purposes only and were not corrected for multiple comparisons. Integral and trabecular volumetric BMD increased more over 12 months' treatment with ibandronate than placebo in the total hip (integral, 1.5% ± 3.4% vs -1.0% ± 2.5%; trabecular, 2.6% ± 8.0% vs -2.9% ± 5.1%), trochanter (integral, 2.5% ± 4.2% vs -1.0% ± 3.4%), and femoral neck (integral, 0.8% ± 3.2% vs -1.4% ± 2.5%). Cortical volumetric BMD changes from baseline in these regions were similar between the groups. The treatment difference in the primary endpoint, integral BMD of the total hip (least-squares means and 95% confidence intervals (CI), ibandronate minus placebo, 2.2% [0.7%-3.7%], *P* = 0.005) showed a clinically meaningful ibandronate effect. FEA modeling of femoral strength under falling load showed that ibandronate improved strength of the whole proximal femur (treatment difference, 5.9%; 95% CI, 2.7%-9.0%, *P* = 0.0004) and the pericortical (treatment difference, 2.5%; 95% CI, 0.6%-4.5%, *P* = 0.0113) and trabecular (treatment difference, 3.5%; 95% CI, 1.3%-5.7%, *P* = 0.0025) compartments. Once-monthly oral ibandronate for 12 months resulted in integral and trabecular volumetric BMD gains in the proximal femur. FEA modeling demonstrated that ibandronate also improved femoral strength with an increase in pericortical as well as trabecular compartment strength.

***Disclosures:* A. Kivitz**, *Roche, GlaxoSmithKline 1.*
This study received funding from: Roche, GlaxoSmithKline.

SA510

Bone Tissue Quality Is Differently Altered by PTH, Bisphosphonates and SERMs. T. C. Brennan*, R. Rizzoli, P. Ammann. Faculty of Medicine, Division of Bone Diseases, Geneva University Hostpital, Genève, Switzerland.

Bone strength, a determinant of resistance to fracture, depends on bone mineral density, bone geometry, microarchitecture, bone turnover rate and intrinsic bone tissue quality. Despite comparable antifracture efficacy, bone resorption inhibitors and bone anabolic agents are likely to modify the various determinants of bone strength in different ways. To address this hypothesis, we treated 8-month old osteoporotic ovariectomized (OVX) rats with pamidronate (APD, 0.6mg/kg 5 days/month s.c.), PTH(1-34) (10 ug/day, s.c.), raloxifene (3 mg/kg 5/7 days) or vehicle for 16 weeks, and measured maximal load and other biomechanical properties with an axial compression test, areal BMD, microarchitecture by microCT, and intrinsic bone quality by nanoindentation. Markers of bone turnover, deoxypyridinoline (Dpd) and osteocalcin, were also determined. Results are Means ± SEM, with significances tested by ANOVA.

Vertebral body	Sham	OVX	APD/OVX	PTH (1-34)/OVX	Raloxifene /OVX
Maximal Load (N)	303.5 ± 27.3**	215.8 ± 11.3	321.1 ± 18.1***	404.9 ± 25.0***	261.9 ± 21.7
BV/TV (%)	28.4 ± 1.25***	18.3 ± 0.87	23.3 ± 1.13**	36.4 ± 1.77***	22.1 ± 1.06
Total Energy (mN*nm)	4265.2 ± 84.8*	3891.7 ± 68.4	4160.6 ± 136.0	3578.4 ± 49.0###	4283.2 ± 62.9*
Osteocalcin (ng/ml)	11.1 ± 0.68	14.9 ± 1.30	8.23 ± 0.05***	23.4 ± 1.21***	12.3 ± 1.71

(***p<0.001, **p<0.01 and *p<0.05 compared to OVX vehicle or ###p<0.001 compared to Sham)
All treatments improved maximal load compared to OVX animals, although the difference was not significant in raloxifene-treated rats. The PTH-induced increase in maximal load was associated with improved microarchitecture, including marked increases in trabecular bone volume and connectivity, leading to values higher than in sham. APD corrected the effects of OVX on trabecular bone volume. Raloxifene had no effect. All treatments increased areal BMD, with potency: PTH>APD>raloxifene. PTH was ineffective in correcting the steep decreases in tissue hardness and working energy caused by OVX. In contrast, APD normalized bone strength, together with a reduced bone turnover and increased hardness. Raloxifene also improved hardness, elastic modulus and markedly enhanced working energy, with low levels of bone turnover markers.
These results demonstrate that inhibitors of bone resorption and stimulators of bone formation reduce fracture risk though different mechanisms. PTH markedly improved bone mass and micro-architecture yet lead to poor trabecular bone quality and increased bone turnover. APD and raloxifene improved bone mass and positively influenced intrinsic bone tissue quality. Through a reduced bone turnover these agents prevented further degradation of mechanical properties.

***Disclosures:* T.C. Brennan**, *None.*

SA511

Development of An Animal Model of Bisphosphonate-Induced Severe Suppression of Bone Turnover. J. E. Zerwekh[1], W. Geng*[1], C. T. Skurla*[2], C. V. Odvina[1]. [1]Center for Mineral Metabolism and Clinical Research, University of Texas Southwestern Medical Center at Dallas, Dallas, TX, USA, [2]Department of Mechanical Engineering, Baylor University, Waco, TX, USA.

There is a growing body of clinical evidence supporting the development of bisphosphonate-induced severe suppression of bone turnover (SSBT) in some patients taking bisphosphonates singly or in combination with other antireorptive agents. We undertook the development of an animal model of SSBT that recapitulated the histological, biochemical, and biomechanical findings in humans. Twelve ovariectomized adult female Sprague-Dawley rats were allowed to acclimatize to their housing. After 2 weeks, rats were placed in individual metabolic cages and urine collected for 3 subsequent days. Under anesthesia, BMD was assessed by Hologic DEXA, and a blood sample obtained. One-half of the rats then received weekly i.p. injections of alendronate at a dose of 35ug/kg for four months, a dosing regimen calculated to expose the skeleton to the same amount of alendronate obtained from weekly 70 mg oral dosing for a total of 10 years. Control rats received saline. At the end of 4 months, all baseline procedures were repeated. Two animals in the control group died following baseline examination. At sacrifice, one femur was obtained for dynamic histomorphometry and determination of osteocyte density. The contralateral femur was procured for biomechanical testing. Compared to control rats, alendronate-treated rats demonstrated a greater increase in spine BMD (30.3 ± 5.2 SEM vs 12.7 ± 3.6%, p=0.028) and significant reductions in bone turnover as determined from histomorphometry. The most striking finding was the total lack of any identifiable tetracycline double label in cancellous bone compared to controls (4.5 ± 2.1%). In addition, alendronate-treated rats had significantly lower viable osteocyte number and an increased number of empty lacunae compared to controls (238 ± 29 vs 85 ± 16/mm^2, p=0.004) Biomechanical testing of femurs demonstrated no significant differences between groups for stiffness or maximum flexural strength. However, alendronate-treated rats demonstrated a significant decline in yield strength of the femur compared to controls (69 ± 9 vs 53 ± 11 MPa, p=0.040). Taken together, these findings are consistent with an alendronate-induced suppression of bone turnover characterized by a lack of bone formation, an increase in the number of empty cortical and cancellous osteocytic lacunae, and reduced biomechanical competence as shown by a significant reduction in the yield point. This animal model of bisphosphonate-induced SSBT will be useful to begin to unravel the complex effects of long-term bisphosphonate use on bone cells and biomechanical integrity.

***Disclosures:* J.E. Zerwekh**, *None.*
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SA512

Effects of Resorption Cavities on Strength of Trabecular Bone. S. K. Easley*[1], D. Shindich*[1], C. J. Hernandez[2], T. M. Keaveny[1]. [1]Mechanical Engineering, University of California, Berkeley, Berkeley, CA, USA, [2]Mechanical and Aerospace Engineering, Case Western Reserve University, Cleveland, OH, USA.

Understanding the relation between bone strength and presence of microcavites should provide insight into how osteoporosis and treatments might alter bone quality. While it has been shown that simulated suppression of microcavities in low-density vertebral bone can in theory improve bone quality, commensurate improvements in bone quality have not been observed due to antiresorptive therapy when applied to high-density canine bone. To understand this discrepancy, we sought to quantify the effect of adding cavities to both low-density and high-density bone on strength after accounting for differences in bone volume fraction (BV/TV). Trabecular bone from the human femoral neck (n=14, age: 70.4 ± 10.1, BV/TV: 22.5 ± 5.9%) and the vertebral body (n=16, age: 72.9 ± 11.7, BV/TV: 11.2 ± 3.7%) were scanned with micro-CT at 22-micron voxel size. Each image was converted into three finite element (FE) models, using the original image of bone or using images in which cavities (44 microns deep) were digitally added to the bone surface either at random (non-targeted) or in regions of greatest maximum principal strain (targeted). FE analysis was used to compute the compressive strength of each specimen, in its intact and altered states. Results indicated that the presence of cavities deleteriously altered the strength-density relationship as compared to models without cavities (p<.001). Strength change per unit percent change in BV/TV due to the addition of non-targeted cavities (1.87 ± 0.23 MPa/%BV/TV) was two times larger than expected from changes in BV/TV alone (0.91 MPa/%BV/TV). The dependency of percent reduction in strength associated with introducing non-targeted cavities (17.1 ± 2.25) on BV/TV was negligible for the pooled data, while strength reduction associated with targeted cavities was negatively correlated with BV/TV (p=.0012), such that at high densities, there was a smaller difference between non-targeted and targeted models. This study indicates that remodeling cavities can have a disproportionate biomechanical effect in high-density bone as well as low-density bone. However, our prior work on high-dose risedronate treatment of dogs (Eswaran, JBMR 2006) revealed no change in the strength-BV/TV relation compared to untreated controls, using the same type of FE analysis techniques as used here on the unmodified micro-CT scans. We conclude that further research is required to determine the conditions (cavity size, drug treatment, skeletal region), if any, under which alteration of the remodeling space might alter bone strength.

***Disclosures:* S.K. Easley**, *None.*
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SA513

Alteration of Trabecular Bone Architecture Following Sciatic Denervation and Subsequent Reinnervation in Rat Proximal Tibiae. H. Tamaki[1], K. Yotani*[1], A. Yuki*[1], I. Sakashita*[1], K. Kitada*[2], H. Kirimoto*[3], F. Ogita*[1], H. Takekura*[1]. [1]Department of Physiological Sciences, National Institute of Fitness and Sports, Kanoya, Japan, [2]Ishikawa National College of Technology, Kanazawa, Japan, [3]Niigata University of Health and Welfare, Niigata, Japan.

Disused rat hindlimb caused by sciatic denervation is characterized by osteopenia accompanying alterations in trabecular bone architecture. We studied the effects of short-term denervation followed by reinnervation on the 2-dimensional architecture of trabecular bone using a unilateral sciatic nerve freezing model rat of temporary disuse. Male Fischer-344 rats aged 11-weeks underwent unilateral hind-limb denervation by either sciatic neurectomy (SN) or nerve freezing (NF) by contact with a stainless steel rod cooled in liquid nitrogen, while control rats were sham-operated. Right and left tibiae of denervated and control rats were obtained at 0, 1, 2, 3, 4 and 5 weeks after surgery. After fixation with a mixture of 1% glutaraldehyde, 1% formaldehyde and 0.05% $CaCl_2$ in 0.1M sodium cacodylate buffer (pH. 7.3), the tibiae were demineralized in 0.1M disodium ethylenediaminetetraacetic acid (pH 7.3) for 6 weeks at 4˚C, dehydrated through a graded ethanol series, and then embedded in paraffin. Histomorphometric analyses were performed on longitudinal sections of proximal tibial metaphyseal secondary spongiosa. Sciatic denervation by SN or NF resulted in a marked loss of trabecular bone, mostly within first 2 weeks after denervation. Trabecular bone area decreased and gradually recovered with the breaking point at 3 weeks, returning to approximately 55% of basal-control levels (at 0 weeks) by 5 weeks after NF. Both the thickness and length of trabecular bone were significantly decreased after denervation. Trabecular thickness at 5 weeks after NF was significantly greater compared to that at 3 weeks after NF and at 5 weeks after SN, while decreased trabecular length after NF did not during the experimental period. These findings suggest that 1) sciatic nerve freezing results in marked loss of trabecular bone, mostly within the first 2 weeks after surgery; 2) temporary denervation and subsequent reinnervation reversibly affects trabecular bone architecture, particularly trabecular thickness.

***Disclosures:** **H. Tamaki**, None.*
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SA514

Beta-Adrenergic Receptor Agonist Administration During Hindlimb Unloading Effectively Mitigates Reductions in Cancellous Bone Formation. J. M. Swift[1], S. N. Swift[2], S. A. Bloomfield[2]. [1]Health and Kinesiology, Texas A&M University, College Station, TX, USA, [2]Health and Kinesiology, Intercollegiate Faculty of Nutrition, Texas A&M University, College Station, TX, USA.

Significant losses in bone mass during extended duration spaceflight pose a major challenge to manned exploration. One ground-based technique for effectively modeling cardiovascular and skeletal effects of spaceflight is rodent hindlimb unloading (HU). HU significantly decreases bone formation rate (BFR/BS) at the proximal tibia metaphysis (PTM) and blood flow to long bone metaphyses. In addition, our lab has demonstrated that dobutamine (DOB), a beta-adrenergic receptor agonist (ADRB), prevents significant declines in PTM total volumetric bone mineral density (vBMD) during HU. The purpose of this study was to assess the effectiveness of DOB in attenuating HU-associated decrements in static and dynamic histomorphometric properties at the PTM. Six-month-old male Sprague-Dawley rats were randomly assigned to cage control (CC) or HU groups (n=24/group). Half of each group was given one daily injection (4 mg/kg BW/d) of DOB (n=12) or an equal volume of saline (VEH; n=12). Two and nine days prior to sacrifice, animals were given subcutaneous doses of calcein (25 mg/kg). After 28 days of HU, undemineralized proximal tibiae were processed for histomorphometric analyses. The 4µm sections (Von Kossa and tetrachrome counter-stained) were analyzed for metaphyseal bone volume (BV/TV), osteoblast surface (Ob.S/BS), osteoclast surface (Oc.S/BS), and trabecular thickness, number and separation (Tb.Th, Tb.N, Tb.S). Unstained 8µm sections quantified mineralizing surface (MS/BS), mineral apposition rate (MAR), and bone formation rate (BFR/BS). At the PTM, HU resulted in no significant changes in BV/TV, Ob.S/BS, Oc.S/BS, Tb.Th, Tb.N, and Tb.Sp. HU did result in significant reductions of MS/BS (-46%), MAR (-25%), and BFR/BS (-58%; $p<0.01$)). However, HU animals treated with DOB had significantly higher MS/BS (105%), MAR (35%), and BFR (168%) than their VEH-treated counterparts ($p<0.01$). No significant differences were detected for these values in the CC rats given DOB vs. CC-VEH ($p>0.05$). These results, in combination with previous *in vivo* peripheral quantitative computed tomography data, suggest that DOB administration during HU prevents significant declines in total vBMD at PTM by mitigating associated decrements in BFR/BS. Future work will determine if these positive effects of ADRB administration are due to enhanced blood flow to the unweighted limbs.

***Disclosures:** **J.M. Swift**, None.*
This study received funding from: The National Space Biomedical Research Institute through NASA NCC 9-58.

SA515

Significant Trabecular Bone Degradation Occurs within Five Days of Muscle Paralysis. S. L. Poliachik*, S. D. Bain, S. Srinivasan, B. J. Ausk*, D. Threet*, P. Huber*, T. S. Gross. Orthopaedics and Sports Medicine, University of Washington, Seattle, WA, USA.

Previously, we have shown that transient muscle paralysis induced by Botox leads to significant bone loss within 3 wk. Furthermore, we have shown that electrical stimulation of the paralyzed muscle fails to prevent this bone loss. The inability of electrical stimulation to prevent this bone loss led us to hypothesize that muscle paralysis leads to an irreversible activation of osteoclastogenesis that cannot be overcome by electrical stimulation intervention. To investigate this hypothesis, we utilized high-resolution microCT to assess the longitudinal changes in trabecular bone of the proximal tibia following transient calf paralysis.
Experimentally, high-resolution microCT images spanning a 2 mm section of the proximal tibia metaphysis were obtained for 28 female C57BL/6 mice (16 wk) immediately prior to injection of Botox into the calf muscle group (2U/100g, d 0). During the course of the study, groups of mice (n = 4) were scanned every other day, beginning d 3 after Botox injection, and ending on d 17 (maximum of 3 live scans per mouse, interim scans separated by 8 d; each total scan time lasting less than 35 min). All mice were sacrificed and a final scan was performed on d 21. Standard image analysis procedures were used to determine trabecular bone parameters for each scan, while ANOVA with multiple comparisons was used to define temporal differences across groups.
Profound degradation of BV/TV was evident within 5 d (-58.1%, $p<0.001$) after calf paralysis and a maximum loss of BV/TV was observed at d 11 (-75.3%, $p<0.001$). While the greatest trabecular bone loss occurred in the proximal tibia metaphysis by d 11, some recovery of BV/TV was evident by d 21 (+23.8% vs. d 11; -51.5% vs. d 0). Maximal decrease in trabecular thickness (-20.03%, $p=0.018$) was observed at d.11 and trabecular number (-22.4%, $p=0.007$) at d 15, with upturn evident by d 21 (+13.1% vs. d 11; +12.7% vs. d 15, respectively).
The magnitude and rapidity of trabecular bone loss observed in this study was surprising, and is similar in magnitude and time course to pathological bone resorption that is induced by pharmacological doses of Vitamin D or PTH. The rapid nature of the skeleton's response to muscle paralysis may also explain, at least in part, the relative ineffectiveness of external muscle stimulation in the treatment of spinal cord injury patients. Further investigations in this model will provide a critical context from which to explore activating mechanisms of osteoclastogenesis, and the apparent role of normal muscle function as an active mediator of skeletal homeostasis.

***Disclosures:** **S.L. Poliachik**, None.*
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SA516

See Friday Plenary number F516.

SA517

Musculoskeletal Disuse Worsens the Acute Detrimental Effects of Heavy Particle Radiation on Osteoblastogenesis. K. Yumoto*[1], R. Mojarrab*[1], J. Arakaki*[1], A. Wang*[1], D. Hilton*[1], E. A. C. Almeida*[1], C. Limoli*[2], N. D. Searby*[1], R. K. Globus[1]. [1]NASA Ames Research Center, Moffett Field, CA, USA, [2]Radiation Oncology, University of California Irvine, Irvine, CA, USA.

Space presents many challenges to human health, including radiation exposure and musculoskeletal disuse. Astronauts lose bone in long duration missions and recovery after return to earth occurs slowly. Although the detrimental effects of disuse on bone are clear, we do not know how the cells needed for skeletal recovery from spaceflight are affected by space-relevant radiation either alone or in combination with musculoskeletal disuse. To test this, 16 week old, male C57Bl/6 mice were either continuously hind limb unloaded (HU) or normally loaded (NL), then irradiated (IR) four days later with Fe-56 (1GeV at 0, 0.1, 0.5 or 2.0 Gy) at the NASA Space Radiation Laboratory (Brookhaven National Laboratory). Tissues were harvested after three more days (total time of HU was 7 days). Bone marrow cells (BMCs) were flushed from femora, counted, plated at constant density, and grown ex vivo with osteogenic or osteoclastogenic additives. Cultures were analyzed for osteoblastogenesis (alkaline phosphatase, ALP- positive colonies and enzymatic activity, DNA content as surrogate for cell number) or for osteoclastogenesis (count of multinucleated TRAP-positive cells). Proximal tibiae were analyzed by microcomputed tomography to assess cancellous structure. IR dose-dependently reduced the numbers of marrow cells freshly harvested from bone but HU had no effect. All the groups demonstrated comparable osteoclastogenesis. IR of NL mice had no effect on growth of osteogenic cells ex vivo but did inhibit osteoblastogenesis (74, 80 and 53% relative to controls with 0.1, 0.5 and 2 Gy, respectively). HU alone also inhibited osteoblastogenesis (75% of controls). Together, IR and HU had a greater effect than either treatment alone; HU plus 0.5 Gy IR showed the lowest ALP activity (41% of controls). Despite the short duration of this study, IR with only 0.1 Gy caused a 16% reduction in cancellous bone volume fraction and 21% decrease in connectivity compared to control. HU did not have an additional effect on the acute bone loss caused by IR. In sum, low doses of heavy particle radiation caused a rapid decline in cancellous bone volume fraction and inhibited osteoblastogenesis while musculoskeletal disuse worsened the inhibitory effects of radiation on osteoblastogenesis. We hypothesize musculoskeletal disuse sensitizes bone marrow osteoprogenitors to radiation and speculate that radiation exposure in microgravity can impair skeletal recovery from disuse.

***Disclosures:** **K. Yumoto**, None.*
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SA518

Effects of Running Exercise on Bone Quality After Immobilization. Z. Peng[1], H. K. Väänänen[2]. [1]Pharmatest Services Ltd, Turku, Finland, [2]Department of Anatomy, Institute of Biomedicine, University of Turku, Turku, Finland.

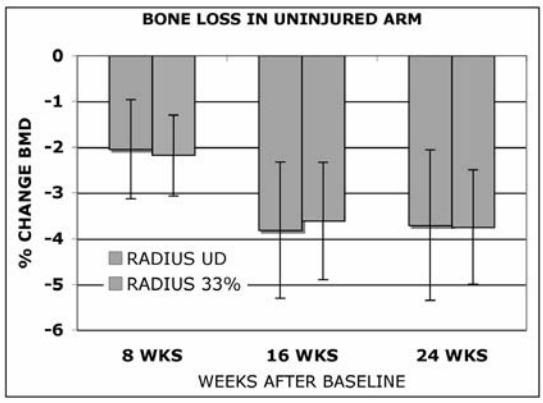
Previous studies have shown that exercise improves mechanical properties of bone in growing rats. The aim of the present study was to investigate whether running exercise can improve bone quality after immobilization. Sixty-seven 10-week-old Sprague-Dawley male rats were used in this experiment. The right hind legs of 59 animals were immobilized with plaster cast in plantar flexion for 3 weeks. After removing the casts, the animals were divided into free activity and treadmill running groups (10 m/min for 30 min/day, 5 days/ week). Tetracycline and calcein were injected 6 and 2 days before sacrifice, respectively. Bone samples were collected from the immobilized legs and the non-immobilized control legs of each animal, and analyzed at 0, 3, 5, 7 and 11 weeks with peripheral quantitative computed tomography (pQCT), histomorphometry and mechanical testing. Immobilization inhibited longitudinal growth of the femur and decreased cortical bone mineral density (BMD), trabecular bone volume (TBV) and tibia ash weight. Running exercise helped to restore the cortical BMD and TBV of the immobilized leg to the level of the non-immobilized control leg. Exercise also increased the growth rate of the immobilized leg and the length of its femur. Three weeks immobilization decreased cantilever bending strength of the femoral neck (FN), but not the three point bending strength of the tibial diaphysis (TD). However, after removing the casts, the strength of the TD was significantly lower in the immobilized leg compared with the control leg. The running exercise was not able to improve the strengths of the FN and TD. Alterations of the cross sectional moment of inertia resulted in different bending strengths of TD between the two legs, and suggested that the alteration of the bone structural properties were related to the pressure of muscles on the bone tissues. Although running exercise significantly increased bone formation rate in the immobilized legs compared with the control legs at the end of the experiment, changes in stress-strain of the tibia revealed that immobilization decreased plastic properties of the bone material. These results demonstrate that running exercise can accelerate the restoration of bone after immobilization, and that a longer than 3-week immobilization may induce irreversible functional decreases in bone quality.

Disclosures: ***Z. Peng****, None.*

SA519

Bilateral Bone Density Loss after Unilateral Disuse in the Forearm. J. A. Spadaro, W. H. Short*, P. R. Sheehe*, R. M. Hickman*. Orthopedic Surgery, Upstate Medical University, Syracuse, NY, USA.

An opportunity arose to determine if changes occurred in the contralateral forearm of subjects with BMD losses due to injury-initiated disuse in one forearm in data from the e-Bone study. We, like many others, assumed that the "normal" forearm would either remain unchanged or perhaps experience a slight BMD increase due to some increased use.
The e-Bone study was a randomized, double blind, sham controlled trial approved by the IRBPHS at SUNY Upstate Medical University (ASBMR 2007 T430). Entry (baseline) was 6-8 weeks after a distal radius fracture or carpal surgery and 99 subjects were randomized to wear a distal forearm PEMF transducer or sham device, 1-4 hours per day for 8 weeks on their involved forearm. Bone mineral density (BMD) and geometry at several sites in both forearms were measured by DXA and peripheral CT at baseline, 8, 16, and 24 weeks. Complete data was available in 82 subjects. Bone formation marker (BSAP) was unchanged on average at 8 weeks vs. baseline, while the resorption marker (CTx) was decreased. In the immobilized forearm, BMD losses by DXA were 5-7 % in the ultra-distal region and 3-4% in the midshaft ($p<0.005$). Since no detectable changes were observed with PEMF, we analyzed the entire group of subjects for changes in the contralateral forearm.
Results showed a statistically significant and substantial BMD loss in the contralateral forearm on average, despite inter-subject variability. The loss tended to increase over the observation period to 24 weeks after baseline, as has been typically observed for an injured extremity (Fig. 1) . The magnitude of loss was about half of that in the injured side at the ultra-distal site (2-4%), but similar to the injured side at the radial shaft site (2-4%).
We hypothesize that this contralateral loss is due to either: (a) systemic hyper-resorptive effects from the injury and/or 'disuse' of the injured side, or (b) may be related to a generalized lower activity level after a forearm injury. This phenomenon needs further exploration and suggests caution in using the uninjured limb as control when studying unilateral BMD changes. (Supported by NIH, NIAMS.)



Disclosures: ***J.A. Spadaro****, None.*

SA520

Immediate Abnormalities at the Chondro-Osseous Junction due to Severe Spinal Cord Injury in Growing Rats. L. R. Morse*[1], B. Solomon*[2], P. Stashenko*[2], R. A. Battaglino[2]. [1]Physical Medicine and Rehabilitation, Harvard Medical School, Boston, MA, USA, [2]Cytokine Biology, Forsyth Institute, Boston, MA, USA.

Spinal cord injury is associated with rapid bone loss and arrested long bone growth by a mechanism that is poorly understood. In this study we sought to determine the effects of severe T10 contusion spinal cord injury (SCI) on the chondro-osseous junction in adolescent rats. A severe lower thoracic (vertebral T10) spinal cord injury was generated by weight drop contusion (10g x 50mm). Severely injured and body weight-matched naïve (no surgery, n=4) male Sprague-Dawley (SD) rats were studied. At 2 days post-injury, hindlimb functional deficits, bone mineral density assessment by PIXImus scan, histological analysis of the distal femoral metaphysis, and immunohistochemical staining for Substance P were performed. At 2 days post-injury, we observed severe hindlimb functional deficits typical of this model. We detected an increase in trabecular osteoclast activation, a decrease in hypertrophic chondrocytes per column of growth plate, a reduction in growth plate width, and an increase in bone levels of Substance P in the injured animals. We demonstrated a rapid activation of trabecular osteoclasts and growth plate arrest due to loss of hypertrophic chondrocytes following contusion spinal cord injury in rodents. These changes are associated with increased substance P expression suggesting that this neurotransmitter may be involved in the mechanism of bone loss and long bone growth arrest following spinal cord injury. If confirmed, Substance P is a potential therapeutic target to alleviate the bony sequelae of spinal cord injury in humans.

Disclosures: ***R.A. Battaglino****, None.*

SA521

Effects of Simulated Resistive Exercise on Cortical Bone in the Tibia Mid-Diapyhsis of Hindlimb Unloaded Rats. H. A. Hogan[1], J. M. Swift[2], M. I. Nilsson*[2], S. A. Bloomfield[2]. [1]Mechanical Engineering, Texas A&M University, College Station, TX, USA, [2]Health and Kinesiology, Texas A&M University, College Station, TX, USA.

The objective of this study was to characterize the effects of controlled electrical muscle stimulation on cortical bone in the tibia mid-diaphysis of hindlimb unloaded (HU) adult male rats. This same general muscle stimulation procedure has been used successfully previously as a countermeasure, but the focus has been on cancellous bone in the proximal tibia metaphysis (PTM). Previous studies showed dramatic anabolic effects in the PTM using eccentric muscle contractions of either 500ms or 1000ms duration. In order to more closely simulate actual resistive exercise in the current study, the protocol used a combination of 1000ms of isometric contraction followed by 1000ms of eccentric contraction. Six-mo. male Sprague-Dawley rats were randomly assigned to cage control (CC; n=12), hindlimb unloading plus muscle stimulation (HU+STIM; n=10), or HU plus anesthesia as another control (HU+AN; n=6) groups. The HU+STIM animals were anesthetized and subjected to muscle stimulation every other day (4 sets of 5 reps) for the 28 days of HU, while HU+AN animals were anesthetized without stimulation following the same schedule. In vivo peripheral quantitative computed tomography (pQCT) scans were taken at the tibial mid-diaphysis on anesthetized animals on days 0 and 28 to assess bone properties. Simulated exercise had a beneficial effect on both BMC and vBMD. The pre-to-post change for HU+STIM animals was +9% for BMC and +5% for vBMD, which are similar to results from the 500ms and 1000ms protocols in previous studies. There was no change for HU+AN, and an 8% increase for CC animals. In addition, tibiae were excised post-sacrifice for determination of mechanical properties at the mid-diaphysis using 3-point bending. Surprisingly, simulated exercise had little effect on mechanical properties. Comparing endpoint values (28d) between HU+STIM and HU+AN, the ultimate load was essentially the same, and there was a trend (non-significant) toward lower stiffness, elastic modulus, and yield stress (~5%). The only dramatic difference was for the total energy absorbed, or work-to-fracture, which was 34% higher for HU+STIM. We speculate that the lack of a beneficial effect on most mechanical properties may be due to differences in the organic matrix, perhaps less mature due to cross-sectional remodeling. Another possible reason may be the relatively low number of animals for biomechanics.

Disclosures: ***H.A. Hogan****, NSBRI 3.*
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SA522

Mechanical Strain Prevents Adipogenesis in Mesenchymal Stem Cells By Stimulating a Durable β-Catenin Signal. B. Sen*[1], Z. Xie*[1], N. Case[1], M. Ma*[1], C. Rubin[2], J. Rubin[1]. [1]Medicine, UNC-CH, Chapel Hill, NC, USA, [2]Biomedical Engineering, SUNY, Stony Brook, NY, USA.

The ability of exercise to decrease fat mass and increase bone mass may arise through mechanical biasing of mesenchymal stem cell (MSC) commitment towards osteoblastogenesis and away from adipogenesis. To study the effect of mechanical strain on lineage selection we ascertained that C3H10T1/2 MSC cultured in a highly adipogenic "A" medium contained lipid at 3 days, and nearly all cells stained for oil-red-O after 5 days. PPARγ and adiponectin mRNA (RT-PCR) and protein (Western) was present at 3d and continued to rise at 5d. Active and total β-catenin levels fell sharply over the 5 d of adipogenesis. Application of strain daily (3600 cycles, 2%) inhibited adipocyte differentiation as measured by decreased cellular lipid droplets at 3 and 5d. Strain as well inhibited expression of PPARγ mRNA by nearly 50%, and

adiponectin by 75%, changes were confirmed by Western. The decrease in β-catenin seen during adipogenesis was entirely prevented by daily application of strain. Cyclin D1 expression reflected the change in β-catenin: cyclin D1 mRNA fell to 51±5% after 5 d in A medium compared to cells in growth medium. Mechanical challenge increased cyclin D1 mRNA to 195 ± 23% at 3d, and nearly 3-fold at 5d ($p<0.01$) compared to unstrained cells in A medium. Expression of second β-catenin response gene, WISP1, paralleled that of cyclin D1: WISP1 dropped to 21±1% by 5d in A medium ($p<0.05$), and increased with mechanical strain to 245 ± 46% and 611 ± 116% after 3d and 5d respectively ($p<0.05$ for both). LiCl similarly prevented adipogenesis, implicating β-catenin in strain's ability to inhibit adipogenesis. Mechanical strain activated AKT followed by inactivation of GSK3β, demonstrated by increase in phospho-forms of both kinases after strain, suggesting a possible pathway for mechanical control of β-catenin activity. Finally, in cultures subjected to mechanical strain, there was enhanced potential for entry into the osteoblast lineage: cells were cultured in A medium ± strain for 3 d, followed by 2 d of BMP2 treatment to induce osteoblast differentiation. In unstrained cultures, Runx2 and osterix did not rise in response to BMP2, but in cultures subjected to mechanical strain, BMP2 increased expression of Runx2 by 40% and osterix by 68%. These results indicate that MSC commitment to adipogenic lineage can be suppressed by mechanical signals even during highly adipogenic conditions, and implicate β-catenin activation as a mechanism. Further, by preserving β-catenin levels, mechanical strain allows MSC to be more responsive to osteogenic factors. Our work suggests that exercise should have positive effects for both osteoporosis and obesity by affecting MSC lineage allocation.

Disclosures: ***J. Rubin****, None.*
This study received funding from: NIAMS.

SA523

See Friday Plenary number F523

SA524

The Effects of Electrical Stimulation on Nitric Oxide Expression and Osteocyte Viability in Ovariectomized Rats. A. P. R. Lirani-Galvao*[1], P. Chavassieux[2], N. Portero-Muzy*[2], O. L. Silva*[3], C. Bergamaschi*[1], M. Lazaretti-Castro[1], P. D. Delmas[2]. [1]Departmento de Medicina, Universidade Federal de Sao Paulo, Sao Paulo, Brazil, [2]INSERM Unit 831, Université de Lyon, Lyon, France, [3]Bioengenharia, Universidade de Sao Paulo, Sao Paulo, Brazil.

We have previously shown in rats that electrical stimulation (ES) may prevent the increased bone turnover, microarchitecture deterioration and bone loss induced by ovariectomy (OVX) (J. Bone Miner. Res. 22 Suppl1:S447, 2007). Osteocytes have been hypothesized to mediate this effect by releasing biochemical signals such as nitric oxide (NO). The aim of the present study was to investigate the potential role of NO in the response to ES on the osteocyte viability in OVX rats. Sixty rats (200-220g) were divided into 6 groups: SHAM; SHAM treated with 6mg/d of L-NAME, an inhibitor of NO synthase (SHAM-L); ovariectomized (OVX); OVX treated with L-NAME (OVX-L) or subjected to an electrical stimulation (OVX-ES) or both (OVX-L-ES) for 12 weeks.
The expressions of endothelial NO synthase (eNOS) and inducible NOS (iNOS) and osteocyte apoptosis (caspase-3 and TUNEL) were assessed by immunostaining. eNOS and iNOS were similarly expressed in subperiosteal regions of the cortices of metaphysis and diaphysis of tibiae in SHAM group, but they were not detected in OVX and L-NAME treated groups. In OVX-ES group, similar expressions of eNOS and iNOS were detected in cortical bone of tibial diaphysis as in SHAM. When compared to SHAM, OVX significantly increased the percentage of apoptotic cells and empty lacunae ($p<0.05$) but this effect was prevented by ES. SHAM-L and OVX-L showed a diminished percentage of apoptotic osteocytes when compared to SHAM and OVX, respectively. Interestingly, this difference was not observed between OVX-L-ES and OVX-ES.
In conclusion, ES may counteract the effects of OVX on bone tissue in rats through the activation of iNOS and eNOS, preserving the osteocyte viability and secondary acting on bone remodelling to maintain the bone structure and architecture. It was not possible, however, to identify if L-NAME blocks the effects of ES on osteocytes, since their effects on theses cells are similar. Regarding osteocyte viability it seems that the effects of ES and L-NAME are similar. We hypothesized that the decreased percentage of apoptotic osteocytes in groups under L-NAME may be due to bone mechanical stimulation caused by high blood pressure levels induced by NO blockage (Turner et al., Bone 1997, 21:487-90). However, the other results cited above suggest that NO may mediate the effects of ES on bone tissue.

Disclosures: ***A.P.R. Lirani-Galvao****, None.*
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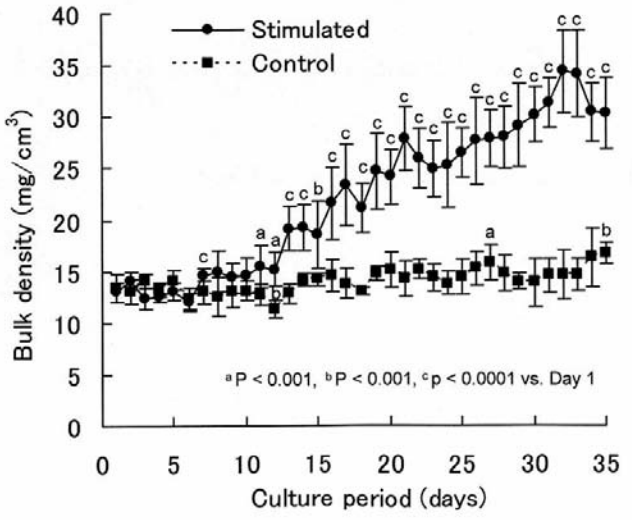
SA525

Strain-Induced Fluid Flow in a Three-Dimensional Porous Matrix Promotes Osteoblastic Calcification *in vitro*. S. M. Tanaka[1], M. Kakio*[2], K. Yamakoshi*[2]. [1]Institute of Nature and Environmental Technology, Kanazawa University, Kanazawa, Ishikawa, Japan, [2]Graduate School of Natural and Science Technology, Kanazawa University, Kanazawa, Ishikawa, Japan.

Bone strain generated by external loading force induces fluid flow in bone matrix, which creates fluid shear stress on bone cells. Strain-induced fluid flow is known to play a dominant role in the regulation of stress-sensitive genes in osteoblasts *in vitro*, compared to substrate strain alone [1]. The purpose of this study is to investigate the capability of strain-induced fluid flow to promote osteoblastic calcification *in vitro*. Osteoblasts differentiated from mesenchymal stem cells in rat bone marrow were seeded into a porous scaffold made of type I collagen (16 mm in length x 20 mm in width x 2 mm in thickness, pore size: about 100 μm) at a cell number of 0.7×10^6 with an osteogenic medium containing 50 μg/ml L-ascorbic acid, 10 mM β-glycerophosphate, and 10^{-8}M dexamethasone. A sinusoidal mechanical load with a magnitude of 2000 μstrain was applied to the scaffold at 0.8 Hz for 3 minutes per day for 35 days using a piezoelectric mechanical stimulator to give cyclic strain-induced fluid flow to the cells in the porous scaffold [1]. The degree of osteoblastic calcification in a scaffold was monitored non-destructively once a day using an optical sensing unit composed of four light emitting diodes (LED) and a photodiode (PD) placed under a culture chamber. The LEDs irradiate a scaffold with near-infrared light at 850 nm increasing its intensity (I_0) and the intensity of diffuse reflectance light (I) from the scaffold is measured by the PD. Using the slope of I_0-I curve, the degree of calcification is evaluated as bulk density (mg/cm^3). In the scaffolds stimulated by strain-induced fluid flow, bulk density started to increase after day 10 and reached finally to about 30 mg/cm^3. On the other hand, the controls without the stimulation did not represent a remarkable increase in bulk density. The results indicated that strain-induced fluid flow could be a potent enhancer for osteogenesis *in vitro*.
1. S.M. Tanaka, et al., Calcif. Tissue Int., 76(4):261-271, 2005.



Disclosures: ***S.M. Tanaka****, None.*
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SA526

Mechanical Loading Upregulates Expression of the Transcription Factor EGR2/Krox-20 by a COX2-mediated Mechanism. G. Zaman*, L. K. Saxon, B. Javaheri*, A. Sunters*, J. S. Price, L. E. Lanyon. Basic Sciences, The Royal Veterinary College, London, United Kingdom.

Microarray analysis of mRNA extracted from the tibiae of 8 female C57BL/6 mice showed differential regulation of 621 genes between bones that had been subjected *in vivo* to a single period of dynamic axial loading (2 Hz for 30 seconds) capable of stimulating osteogenesis, compared with unloaded contra-lateral bones. At 3 hours after loading one of the genes showing substantial (1.9 fold) differential up-regulation was EGR2/Krox-20. Expression levels were restored to those in controls by 8 hours after loading. These results were confirmed by quantitative real-time PCR (qRT-PCR).
In vitro, monolayer cultures of primary osteoblast-like cells prepared from C57BL/6 mouse long bones and UMR106 cells, when subjected to a single period of mechanical strain (peak strain 3400 microstrain, 1 Hz, 600 cycles), showed a maximal 2 and 3 fold increase respectively in EGR2 mRNA levels (by qRT-PCR) 1 hour after exposure to strain. This strain-related increase in EGR2 mRNA levels was abolished by the COX2 inhibitor NS398 (1 μM) and imitated (2.6 fold increase) by exogenous prostaglandin (PG) E2 (5 μM). The PGE2-induced increase in EGR2 expression was abolished by blocking the EP1 & EP2 PG receptors (30 μM AH6809), whereas inhibiting the EP4 receptor inhibitor (30 μM AH23848) only partially lowered expression (by 29%). The PKC agonist PMA (500 nM) caused a significant increase in EGR2 mRNA expression whereas exogenous dibutyryl-cyclic AMP (100 μM), which activates PKA signaling, caused a significant decrease. Consistent with this finding, pretreatment with the PKA antagonist H89 (1 μM) significantly increased PGE2-induced EGR2 expression.
That bone cells respond to mechanical strain by COX2 mediated release of prostanoids has been recognized for some time. The immediate downstream consequences of this PG release have been unclear. The data reported here suggest that one of these downstream targets is EP receptor-mediated increase in the expression of EGR2, a zinc finger transcription factor belonging to the Early Growth Response (EGR) family. EGR2 has previously been reported to be involved in osteoblast differentiation and to act as a pro-survival factor. Suppression of EGR2 expression has been implicated in glucocorticoid-induced osteoporosis and EGR2 mutant mice have defective bone formation. To our knowledge EGR2 has never before been implicated in bone cells' responses to mechanical loading.

Disclosures: ***G. Zaman****, None.*
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SA527

See Friday Plenary number F527.

SA528

Integrin Mediated Mechanical Forces Stimulate Differentiation of Mesenchymal Stem Cells. P. Müller*, A. Kasten*, C. Bergemann*, J. Rychly. Laboratory for Cell Biology, University of Rostock, Rostock, Germany.

Mesenchymal stem cells are multipotent and differentiate into different phenotypes including osteoblasts in dependence on the environment. Mechanical forces play a significant physiological role in the control of cells. Although evidence exists that mechanical forces are a significant factor to drive mesenchchymal stem cells towards the osteogenic differentiation, little is known how physical forces contribute to the decision for a defined differentiation pathway. Concerning the mechanisms, how cells sense mechanical forces, adhesion receptors like integrins function as mechanotransducers and mediate applied forces from the extracellular matrix to the cytoskeleton in the cell interior. Therefore we applied a technique to mechanically stress integrin receptors on the apical surface of adherent cells, using antibody coating magnetic beads. β1-integrin receptors of human bone marrow derived mesenchymal stem cells were mechanically stressed for 15 min with 1 Hz. Scanning electron microscopy revealed that stressing the receptors induced a slight distortion of the cell surface in the vicinity of a bead but had no effect on the global cell shape. Mechanical stress to the integrin receptor induced a signal transduction which was detected by activation of the MAP-kinases ERK 1/2 and the signaling protein Akt. To test the effect of mechanical integrin stress on the differentiation of mesenchymal stem cells, we analysed the expression of characteristic marker proteins for osteogenic and adipogenic differentiation using real-time PCR and Western blot. Mechanical stress induced an increased expression of the transcription factor Runx2 as well as VEGF, collagen I and ALP on the mRNA level within 1 hour after the applied stress. This indicated that integrin mediated mechanical forces induced the osteogenic differentiation of mesenchymal stem cells. Analyses of the expression of PPARγ, a marker for the adipogenic differentiation pathway, revealed a pronounced increase of the mRNA due to mechanical stress after 24 h. Together, the results demonstrated that mechanical integrin stress drives mesenchymal stem cells both to the osteoblastic and adipogenic differentiation. It appeared that expression of osteogenic markers occured faster, whereas proteins related to the adipogenic pathway are stimulated later by mechanical forces.
We conclude that mechanical forces mediated by integrin receptors are able to stimulate the differentiation of mesenchymal stem cells into different directions and the final cellular phenotype will be determined in the context with different other environmental factors.

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SA529

Effect of MKP-1 Deletion on the Fluid Shear Stress Induction of COX-2 Expression in Osteoblasts. L. Ma*[1], M. Mehrotra*[2], S. Choudhary[1], L. Raisz[1], C. Pilbeam[1]. [1]Department of Medicine, University of Connecticut Health Center, Farmington, CT, USA, [2]Department of Pathology and Laboratory Medicine, Medical University of South Carolina, Charleston, SC, USA.

Mitogen activated protein (MAP) kinase phosphatase-1 (MKP-1) can dephosphorylate and inactivate MAP kinases, including extracellular signal-regulated kinase (ERK). ERK activation has been shown to be important for the fluid shear stress (FSS) induction of cyclooxygenase-2 (COX-2), the major enzyme regulating prostaglandin production, in osteoblasts. The goal of this study was to evaluate the role of MKP-1 in the FSS regulation of COX-2. We applied 30 min of FSS (pulsatile, 10 dynes/cm^2) to MC3T3-E1 cells or calvarial osteoblasts, followed by static culture for up to 4 h. Calvarial osteoblasts were sequentially digested from neonatal calvariae of MKP-1 wild type (WT) and knockout (KO) mice, and populations 2-5 were pooled, grown to confluence and replated for experiments. mRNA and protein expression was measured by real-time PCR and Western blot, respectively. In MC3T3-E1 cells, FSS stimulated a 6-fold induction of MKP-1 mRNA and an 8-fold induction of COX-2 mRNA. The induction of both MKP-1 and COX-2 peaked 30 min after cells were returned to static culture (post-FSS). The FSS induction of ERK phosphorylation in MC3T3-E1 cells was biphasic, peaking at 30 min of FSS and decreasing at 30 min to 1 h in post-FSS culture before increasing again at 2-4 h. Treatment with a specific ERK inhibitor PD98059 (50 μM) significantly decreased COX-2 induction. In calvarial osteoblasts from MKP-1 WT and KO mice, the temporal patterns of FSS-stimulated ERK phosphorylation and COX-2 mRNA expression were similar to those seen in MC3T3-E1 cells. However, the FSS-stimulated phosphorylation of ERK was increased in MKP-1 KO osteoblasts at all time points compared to WT osteoblasts. FSS-stimulated COX-2 mRNA expression was also increased in MKP-1 KO osteoblasts compared to WT osteoblasts. These data suggest that FSS-stimulated MKP-1 expression limits the FSS stimulation of both ERK phosphorylation and COX-2 expression. We conclude that FSS-stimulated MKP-1 expression may be important for regulating responses to mechanical loading of bone.

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SA531

Interfacial Tissue Response is Influenced by Local Strain Created During Implant Micromotion. R. M. Wazen*[1], J. B. Brunski*[2], J. A. Currey*[2], J. A. Helms*[3], P. Leucht*[3], A. Nanci*[1]. [1]Faculty of Dentistry, Universite de Montreal, Montreal, QC, Canada, [2]Rensselaer Polytechnic Institute, Troy, NY, USA, [3]Stanford University, Stanford, CA, USA.

During healing of an implant in bone, loading can create implant micromotion at the bone-implant interface. Mechanical conditions and interfacial strain associated with implant micromotion also contribute to the regulation of cell and tissue healing response. Excessive implant micromotion can lead to fibrous encapsulation and implant loosening. The objective of this study was to characterize the influence of interfacial strain in the environment of an implant on bone healing in a mouse. The micromotion system consisted of a miniature device in which pin or screw-shaped implants were installed through a hole drilled in one cortex of the tibia. These implants were placed in holes of different sizes to produce interfaces with (1) no initial contact between implant and bone, and (2) a direct bone-implant contact. Implants were subjected to a displacement of 150 μm at ~1 cycle/sec for 60 cycles/day for a 7 day period. Control implants in both types of interfaces were stabilized throughout the 7-day healing period. Undecalcified tissue sections of control and strained interfaces at 7 days were prepared and stained with Goldner to evaluate tissue reaction in relation to higher strain regions present at the ridges and base of the pin implants, and lower strain regions on the smooth sides of the pins. Experimental strain analyses and finite element simulations were used to characterize interfacial strain fields. In stable implants, normal bone formation occurs consistently around the implants. In implants subjected to micromotion, bone regeneration was disrupted in areas of high strain concentrations (e.g., greater than 30%), whereas lower strain values contributed to bone formation. These results suggest that in the early healing period, high principal tensile and/or compressive strains interfered with normal bone regeneration. It is concluded that implant micromotion as well as interfacial strain field contribute to regulating the interfacial mechanobiology at healing bone-implant interfaces.

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SA532

Bone Resorption and Articular Cartilage Degenerative Changes Following Performance of High Repetition High Force Tasks Are Attenuated by Secondary Ibuprofen Treatment. M. F. Barbe[1], M. Amin*[2], M. Harris*[2], J. B. Driban*[3], F. F. Safadi[4], A. E. Barr*[5]. [1]Physical Therapy; Anat & Cell Biology, Temple University, Philadelphia, PA, USA, [2]Physical Therapy, Temple University, Philadelphia, PA, USA, [3]Kinesiology, Temple University, Philadelphia, PA, USA, [4]Anat & Cell Biology, Temple Medical School, Philadelphia, PA, USA, [5]Physical Therapy, Thomas Jefferson University, Philadelphia, PA, USA.

Using our rat model of voluntary repetitive and forceful reaching, we have shown than high repetition low force tasks result in increased osteoclasts and macrophages in forelimb musculoskeletal tissues. Here, our purpose was to determine if high demand repetitive tasks lead to bone resorption, cartilage damage and increased serum biomarkers of bone and cartilage turnover, and if the mechanism has an inflammatory component. 54 young adult, female Sprague-Dawley rats were trained to perform an upper extremity high force lever pulling task (60% max. force) at high reach rates (12 reaches/min) (HRHF) for 12 weeks; 21 were treated with ibuprofen (oral; 45 mg/kg body weight/day) from the end of wk 4to wk 12 while continuing to perform the task. Results were compared to 12 normal and 9 ibuprofen treated age- and weight-matched controls. After perfusion fixation, micro-CT analysis was used to examine distal forelimb bones from 3 rats/gp. We observed decreased trabecular number and increased trabecular separation in 12 wk HRHF rats (Tb N/mm: 1.75 +/- 0.19 and Tb Sp (um): 0.36 +/- 0.03) compared to controls (Tb N/mm: 2.33 +/- 0.21 and Tb Sp): 0.26 +/- 0.006), changes ameliorated by ibuprofen (Tb N/mm: 2.22 +/- 0.39 and Tb Sp: 0.32 +/- 0.06) ($p<0.01$). Forelimb bones (n=6/gp) were embedded, sectioned, stained immunostained and osteoclasts counted. Osteoclasts increased in distal radius and ulna at the periosteal-bone interface and trabecular surfaces in 12 wk HRHF rats ($p<0.001$). Bones were also stained with Safranin O to examine wrist joint surfaces for cartilage changes. Safranin O losses were observed in the articular cartilages of 12 wk HRHF rats only. Serum from all rats was assessed using ELISA for bone turnover markers (n=6-9/gp). Serum C12C, CTX1 and TRAP5b were significantly increased in the 12 wk HRHF rats (1.5 fold, 1.79 fold and 1.28 fold increases, respectively). Ibuprofen treatment did not ameliorate TRAP5b increases, but reduced C12C, CTX 1 and the ratio of CTX/TRAP5b levels back to control levels ($p=0.04$, $p<0.001$, $p<0.01$, respectively). Thus, performance of the HRHF task for 12 weeks resulted in increased osteoclast numbers and activity, and bone resorption in forelimb bones. These changes were attenuated by secondary intervention with ibuprofen, thus suggesting that these changes are at least partially induced by inflammatory processes.

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SA533

Targeted Exercise against Hip Fragility. R. Nikander[1], P. Kannus*[1], P. Dastidar*[2], M. Hannula*[3], L. Harrison*[2], T. Cervinka*[3], N. G. Narra*[3], R. Aktour*[3], T. Arola*[3], H. Eskola*[3], S. Soimakallio*[4], A. Heinonen[5], J. Hyttinen*[3], H. Sievänen[1]. [1]UKK Institute, Tampere, Finland, [2]Radiology, Regional Medical Imaging Center, Tampere University Hospital, Tampere, Finland, [3]Biomedical Engineering, Tampere University of Technology, Tampere, Finland, [4]Radiology, Regional Imaging Center, Tampere University Hospital, Tampere, Finland, [5]Health Sciences, University of Jyväskylä, Jyväskylä, Finland.

Objectives To investigate whether sports involving either high-magnitude vertical impacts (triplejumping and high-jumping), moderate-magnitude impacts from rapidly varying and odd directions (soccer and squash-playing), high-magnitude muscle forces (power-lifting), low-magnitude impacts at high repetition rate (endurance running), or non-weight bearing muscle activity at high repetition rates (swimming) would strengthen the cortical bone at the proximal femur, and if so, to what extent and at which anatomic regions of the femoral neck.
Design Cross-sectional study
Setting The UKK Institute for Health Promotion Research and Tampere University Hospital, Finland in 2007-2008
Participants 91 adult female athletes, with ~10 years of intense sport-specific training on average and competing actively at the national or international level, and 20 non-athletic female referents
Main outcome measures Segmental cortical thickness at the femoral neck and trochanteric region of the proximal femur as assessed by three-dimensional magnetic resonance imaging of the whole proximal femur.
Results At the inferior segment, only the high-impact group differed significantly (~55%, p=0.012) from the reference group, while at anterior segment, the cortical thickness in both the high-impact and odd-impact group differed from the reference group (~20%, p=0.042 and p=0.044, respectively). Similarly, the posterior cortical wall was ~20% thicker (p=0.014 and p=0.006, respectively) in these two groups. At the superior segment, the cortical thicknesses did not significantly differ between the groups.
Conclusion Long-term high-impact and odd-impact exercise-loading has similar ability to strengthen the femoral neck cortical bone - not only at the primary weight-bearing inferior segment but also at the lateral segments of the femoral neck, considered critical in terms of hip fragility. Since odd-impact exercises are mechanically less demanding to joints, muscles, and bones than vertical high-impact exercises, this type of training, comprising moderate-magnitude impacts from odd directions, is recommended as a feasible basis for devising targeted exercises against hip fragility.

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SA534

Bone Density Comparisons in Young Male Rock Climbers, Weight Lifters and Sedentary Controls. V. D. Sherk, M. G. Bemben*, D. A. Bemben. Health and Exercise Science, University of Oklahoma, Norman, OK, USA.

Technical free rock climbing is a physically demanding activity that has gained popularity in recent years, but it is unclear how climbing affects bone health. The combination of impact loading received on the body when taking a lead or bouldering fall and the dynamic movements required for climbing often mimic calisthenic, resistance training or plyometric exercises, therefore, this recreational activity may have bone loading effects. The purpose of this study was to compare bone density of the total body, AP lumbar spine, dual proximal femur and forearm in advanced rock climbers (RC), resistance trained men (RT), and untrained controls (CTR), ages 18-35 years. Fifteen advanced rock climbers, 16 resistance-trained men, and 16 untrained men volunteers were recruited for the study. Areal bone mineral density (aBMD) and bone mineral content (BMC) were assessed using DXA (GE Lunar Prodigy, enCORE 2006 version 10.50.086). Body composition variables (% body fat, fat mass, bone free lean body mass) were obtained from the total body scan analysis. Subjects also completed questionnaires to assess physical activity (Baecke) and daily calcium intake. No significant ($p > 0.05$) group differences in calcium intake, body weight, or bone free lean body mass were observed. RT (22.3 ± 0.5 yrs) was significantly ($p < 0.05$) younger than other two groups (RC 25. 9± 1.4 years; CTR 26.7 ± 1.3 years). RC reported being more physically active than RT and CTR, and they had a significantly ($p < 0.05$) lower % body fat (14.5 ± 4.8%) and fat mass (10.9 ± 1.2 kg) than RT (19.4 ± 2.2% and 10.9 ± 1.2 kg) and CTR (27.3 ± 2.3% and 23.2 ± 2.6 kg). One-way ANOVA showed that RT had significantly ($p < 0.05$) greater aBMD at the L1-L4 spine and femoral neck than RC and CTR, and RC had significantly lower aBMD at the 33% radius than CTR. After adjusting for fat mass, RT also had significantly ($p < 0.05$) total body BMD than CTR. In conclusion, rock climbers exhibited similar BMD values as controls for the spine and hip sites, however, their mean forearm BMD was approximately 10% lower than controls.

Table 1. Areal Bone Mineral Density Values for Rock Climber (RC), Resistance Trained (RT), and Control (CTR) Subjects. (Means ± SE)

aBMD site (g/cm^2)	RC (n=15)	RT (n=16)	CTR (n=16)
Total Body	1.246 ± 0.024	1.319 ± 0.020	1.268 ± 0.019
L1-L4 AP Spine	1.203 ± 0.031	1.341 ± 0.031[a]	1.217 ± 0.020
Total Hip	1.111 ± 0.033	1.215 ± 0.027	1.142 ± 0.032
Femoral Neck	1.092 ± 0.032	1.267 ± 0.034[a]	1.129 ± 0.035
Trochanter	0.907 ± 0.026	0.966 ± 0.023	0.920 ± 0.035
33% Radius	0.882 ± 0.030[b]	0.954 ± 0.023	0.979 ± 0.018

[a] Significantly greater than RC and CTR, $p < 0.05$; [b] Significantly lower than CTR, $p < 0.05$.

***Disclosures:* V.D. Sherk**, *None.*

SA535

Jumping Mechanography Safely Evaluates Muscle Performance in Older Adults. B. Buehring, S. Valentine*, A. Woods*, M. Checovich, D. Krueger, N. Binkley. Osteoporosis Clinical Research Program, University of Wisconsin, Madison, WI, USA.

Neuromuscular function declines with advancing age and is strongly associated with increased risk for falls, hip fractures and decreased quality of life. A variety of tests, e.g., chair-rise or timed-up-and-go, assess muscle function. However, a need exists for more sensitive tools to evaluate interventions designed to enhance neuromuscular performance. Jumping mechanography quantitatively measures an individual's ability to generate power and correlates with the methods noted above. However, only limited data evaluating the safety of older adults performing maximal countermovement jumps (CJ) on a force platform exists. As such, this study investigated safety and utility of jumping mechanography in 40 (20 men/20 women), community-dwelling adults over age 60. At baseline, jumping mechanography of two-leg CJ was performed on a force platform (Leonardo, Novotec, Pforzheim, Germany). All participants completed three maximal CJ; maximal jump height [cm] and specific power [W/kg] were calculated. Main outcomes were worsening pain and new vertebral fracture. Pain was assessed using a visual analog pain scale before and immediately after jumping and 7 days later. Bone mineral density (BMD) and vertebral fracture assessment (VFA) were performed using a Lunar iDXA densitometer (GE Healthcare, Madison, WI) prior to CJ and VFA was repeated in 7 days. Stadiometer measured height was obtained at baseline and day 7. Data were analyzed using linear regression and t-test. Age [mean (± SD, range)] and BMI were 77 (± 9, 63-91) years and 25.5 (± 3.6, 19.4-34.1) kg/m^2 respectively. Mean lowest T-score of the L1-4 spine, total femur or femur neck was -1.43 (± 1.1; range 2.4 to -3.1). At baseline, 6 participants had prevalent fractures; 4 had multiple fractures. Power and jump height were lower in older volunteers and did not differ by gender. Mean jump height in women was 14.4 cm ± 5.8 and 16.4 cm ± 6.8 in men. Mean power in women was 19.2 W/kg ± 5.6 and 21.1 W/kg ± 6.3 in men. Pain was low at baseline and follow-ups, ranging from 0 to 5. Pain was reported by 8 individuals before jumping; after jumping, 4 reported a pain change of 1, two noted new pain, one worsened and one improved. A week later, compared to baseline, 6 reported worsening pain and 6 improved. No height or vertebral fracture status change was observed after jumping. In summary, jumping mechanography demonstrates lower jump height and generated power with advancing age. Additionally, it is safe with no substantial increase in pain or worsening of vertebral fracture status in older adults, including those with low BMD and prevalent vertebral fracture. Further evaluation of this methodology as a tool to evaluate change in neuromuscular performance of older adults is indicated.

***Disclosures:* B. Buehring**, *None.*

SA536

See Friday Plenary number F536.

SA537

The Influence of Muscle Size and Strength on Changes in Bone Mass and Size During Growth and in Response to Exercise: A Longitudinal Study. G. Ducher[1], R. Daly[2], J. Black*[3], C. Turner*[4], S. Bass[1]. [1]Centre for Physical Activity and Nutrition Research, Deakin University, Burwood, Australia, [2]Department of Medicine, Western Hospital, The University of Melbourne, Melbourne, Australia, [3]Musculoskeletal Research Centre, La Trobe University, Heidelberg, Australia, [4]Biomedical Engineering, Indiana University-Purdue University, Indianapolis, IN, USA.

The objective of the study was to investigate the influence of growth and exercise-related changes in muscle size and strength on the changes in bone mass and geometry.
Fifty-two competitive tennis players (26 boys), mean age 13.7 yrs (range 6.7-16.5 yrs), had their dominant and non-dominant humeri scanned by MRI at baseline and 12 months. Total bone and muscle cross-sectional areas (BCSA and MCSA) were determined from the mid (40-50%) and distal humerus (60-70%). Humeral bone mass (BMC) was derived from the whole body DXA scan and grip strength was assessed using a dynamometer. Annual changes (Δ) were calculated for height and all the aforementioned parameters. Pubertal status was self-assessed using Tanner stages.
Growth effect *(*nondominant humerus): ΔBMC, ΔBCSA, ΔMCSA and Δ grip strength were closely related to the increase in height (r=0.35-0.71, p<0.05-0.0001). Baseline pubertal status was a significant predictor of ΔBMC and ΔBCSA only after adjustment for Δ height (r=0.26-0.40, p<0.01-0.07). Although ΔBMC and ΔBCSA correlated with ΔMCSA (r=0.30-0.55, p<0.005) and Δ grip strength (r=0.33-0.40, p<0.05), hierarchical regression showed that Δ height and baseline pubertal status explained 36-55% of the variance for the ΔBMC and ΔBCSA (p<0.05) and neither ΔMCSA nor Δ grip strength contributed to the model. Exercise effect: The dominant humerus, which was submitted to repetitive loading through tennis playing, showed 44-66% greater ΔBMC and ΔBCSA than the nondominant humerus (p<0.0001). The difference in ΔBMC between both humeri remained significant after adjustment for ΔMCSA or Δ grip strength (p<0.01). This suggests that non-muscular factors contributed to the exercise-induced increase in bone mass and bone size. The average training volume throughout the year (hours/wk) was a significant predictor of ΔBMC and ΔBCSA (r=0.28-0.41, p<0.05), even after adjustment for ΔMCSA. The largest proportion of the variance in ΔBMC was explained by Δ height and baseline pubertal status (33%, p<0.0001) with an extra 9% explained by the average training volume (p=0.009).
Changes in body size affect both muscle and bone tissues, largely explaining the strong associations between changes in bone mass or size and muscle size. Additional loading (tennis playing) stimulates bone accrual independently of changes in muscle size or muscle strength, possibly through repetitive impacts.

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SA538

HSA and Physical Activity in Boys and Girls During Puberty. G. Y. Rochefort*[1], R. El Hage*[2], D. Courteix*[1], E. Lespessailles[1], C. L. Benhamou[1], C. Jacob*[2], C. Jaffré*[1]. [1]Rheumatology, INSERM U658, IPROS, CHR Orleans, Orleans, France, [2]Faculty of Arts and Sound Sciences, Physical Education Division, Tripoli, Libyan Arab Jamahiriya.

Introduction: Physical loading increases bone mass, bone mineral density and bone strength during puberty. During adolescence, sexual dimorphism in bone, lean and fat mass increases, giving rise to greater size and strength of the male skeleton. However, little is known about the gender differences in femoral neck geometry in adolescent athletes.
Methods: 12 adolescent boys and 8 adolescent girls participated in this study (mean age 15.25 +/- 1.07 years). They were all athletes practicing impact sports (mean 8h/week). Body composition, total body, lumbar spine and femoral neck bone mineral content (BMC) and bone mineral density (BMD) were measured by dual-energy X-ray absorptiometry (DXA). Hip Structure Analysis (HSA) software was used to analyze bone densitometry scans. HSA was derived from images acquired from bone mineral scanners at femoral necks. The main structural parameters derived by HSA are the bone cross-sectional area (CSA), the section modulus (Z) and the cross-sectional moment of inertia (CSMI). A bone Strength Index (SI = Z/height) and two related bone strength (RBS_lean and RBS_weight) were calculated to express bone morphology based on known relationships. Physical activity and daily calcium intake were calculated using questionnaires.
Results: Results are presented in table below. Height, weight, lean mass and calcium intake were significantly greater in boys compared to girls. BMC measurements were also greater in boys than in girls. After adjustment by anthropometric parameters all differences disappeared. Cross-sectional area (CSA), section modulus (Z) the cross-sectional moment of inertia (CSMI) and the SI were greater in boys compared to girls. In contrast, SI normalized to lean mass or weight (RBS_lean and RBS_weight) did not differ between the two groups.
Conclusion: These data suggest that the differences between girls and boys at this age seem only bound to anthropometric differences and that the physical activity does not seem to have a sex-specific action on the bone parameters analyzed in this study.

(Mean +/- SD)	Boys (n=12)	Girls (n=8)
BMC (g)	2.57 +/- 0.42	2.02 +/- 0.23
BMD (g/cm2)	1.20 +/- 0.13	1.11 +/- 0.06
CSA (cm2)	4.27 +/- 0.64	3.37 +/- 0.30
CSMI	4.23 +/- 0.76	2.63 +/- 0.41
Z (cm3)	2.34 +/-0.37	1.66 +/- 0.18
SI (Z/height)	7.39 +/- 2.05	7.05 +/- 0.92
RBS_lean	0.025 +/- 0.003	0.026 +/- 0.002
RBS_weight	0.021 +/- 0.002	0.020 +/- 0.002

***Disclosures: G.Y. Rochefort**, None.*

SA539

Bone Volumetric Density, Geometry and Strength in Male and Female Collegiate Runners. A. J. Thieschafer*[1], J. M. Hughes*[1], K. L. Popp*[1], B. Kaufman*[1], R. J. Wetzsteon[2], S. D. Stovitz*[3], M. A. Petit[1]. [1]School of Kinesiology Laboratory of Musculoskeletal Health, University of Minnesota, Minneapolis, MN, USA, [2]Children's Hospital of Philadelphia, Philadelphia, PA, USA, [3]Department of Medicine, University of Minnesota, Minneapolis, MN, USA.

Given the characteristics of the mechanical loads on bone during running, this activity should theoretically have a positive effect on bone strength. However several studies suggest runners have low or normal areal bone mineral density (*a*BMD, g/cm^2) compared to non-running controls. Bone geometry can adapt in ways that improve bone strength with no change in bone density or mass. The purpose of this study was to explore differences in tibial bone geometry, volumetric density (vBMD, mg/mm^3), and estimates of bone strength in runners and healthy, inactive controls. We used peripheral quantitative computed tomography (pQCT, Orthometrix XCT 3000) to assess tibial bone properties in male (n = 21) and female (n = 38) runners and inactive age-matched healthy controls (n = 17 males, 32 females) aged 18-35 (mean 22.6±3.3yrs). We measured vBMD, bone area (ToA, mm^2) and an index of compressive bone strength (bone strength index; BSI, mg^2/mm^4= ToA * ToD^2) at the distal (4%) tibia. At the midshaft sites (50 and 66% tibia) ToA and cortical (CoA, mm^2) bone area, cortical density (CoD, mg/cm^3), cortical thickness (CoTh, mm), estimated bending strength (strength strain index; SSIp, mm^3) and muscle cross-sectional area (MCSA) were assessed. We used analysis of covariance (ANCOVA) adjusting bone outcomes for age, tibia length and body weight. At the distal (4%) tibia, female runners had significantly greater BSI (+19%, $p < 0.05$) due to a larger ToA (+11%, $p < 0.05$), but no difference in vBMD compared to controls. At the proximal sites, female runners also had significantly greater bone strength (SSIp +17-19%, $p < 0.001$) due to a greater ToA (+14%), CoA (+15%), and CTh (+8-9%) but no difference in vBMD compared to female controls. Male runners, compared to controls had significantly greater CTh (+8-14%, $p < 0.05$) at both proximal sites as well as a greater CoA (+11%, $p < 0.009$) at the 66% site, but no differences in bone strength or vBMD. Greater bone strength in female runners was attributable to greater bone area rather than density. In contrast, there was no difference in bone strength between male groups; however male runners had favorable bone geometric properties. These data suggest that running may optimize bone geometry resulting in increased bone strength in females.

***Disclosures: A.J. Thieschafer**, None.*

SA540

Bone Geometry, Strength, and Muscle Mass in Female Distance Runners with a History of Stress Fracture. K. L. Popp*[1], J. M. Hughes*[1], A. J. Thieschafer*[1], S. A. Novotny*[1], S. D. Stovitz*[2], S. Koehler*[3], M. A. Petit[1]. [1]Kinesiology, University of Minnesota, Minneapolis, MN, USA, [2]Medicine, University of Minnesota, Minneapolis, MN, USA, [3]Allina Clinics, Northfield, MN, USA.

Stress fractures are common among distance runners. While stress fractures are associated with low areal bone mineral density, little is known about the correlation of lower extremity stress fractures with both bone structure and muscle mass. The purpose of this study was to determine differences in bone geometry, estimates of bone strength, and muscle size in female runners with and without a history of stress fractures. A total of 39 competitive female distance runners aged 18-35, with (SFX, n = 19) or without (NSFX, n = 20) a history of lower limb stress fracture were recruited for this cross-sectional study. Peripheral Quantitative Computed Tomography (pQCT, Orthometrix XCT 3000) was used to assess volumetric bone mineral density (vBMD, mm^3), bone area (ToA, mm^2), and estimated compressive bone strength (bone strength index; BSI= ToA * ToD^2 (bone density)) at the distal tibia (4%). Total (ToA, mm^2) and cortical (CoA, mm^2= ToA * ToD^2) bone area, cortical density, and estimated bone bending strength (stress strain index; SSIp, mm^3) were measured at the 15%, 25%, 33%, 45%, 50% and 66% sites. Muscle cross sectional area (MCSA) was measured at the 50% and 66% sites. Questionnaires were used to assess health and training history. There were no significant differences in age, BMI, years of training, miles run per week, or age of menarche between groups. Women with a history of SFX had significantly smaller (7 - 8%) CoA at the 45%, 50%, and 66% sites in both the right and left legs ($p < 0.05$ for all). SSIp was significantly lower (9 - 10%) at the 50% and 66% sites. Runners with a history of stress fracture also had smaller MCSA (7 - 8%) at the 66% site in both the legs. The remaining bone parameters, including vBMD were not significantly different between groups. After adjusting for MCSA there were no differences between groups for any measured bone outcomes. These findings suggest that bone CoA, strength and MCSA are all lower in runners with a history of stress fracture. However, the lower bone CoA and strength were adapted to the lower muscle size, suggesting interventions to reduce stress fracture risk might be aimed at improving muscle size and strength.

***Disclosures: K.L. Popp**, None.*

SA541

Previous Sport Activity During Childhood and Adolescence Is Associated with Bone Geometry in Young Adult Men. M. Nilsson*, C. Ohlsson, D. Mellström, M. Lorentzon. Center for Bone Research at the Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden.

Physical activity (PA) during growth has been associated with altered cortical bone geometry, but it remains uncertain if the PA induced increments in cortical bone size remain when the level of PA is diminished or ceased. The aim of this study was to investigate if PA during growth is associated with areal bone mineral density (aBMD) and cortical bone geometry parameters in currently inactive men at the age of peak bone mass (PBM).
In this population-based study, 367 currently physically inactive men, 19.0 ± 0.6 (mean±SD) years old, were included. DXA was used to assess aBMD at several bone sites, whereas cortical bone geometry and trabecular vBMD at the tibia and radius were measured using pQCT. A standardized questionnaire was used to collect information about previous sport activity (SA).
Regression analysis (including covariates age, height, weight, calcium intake, smoking, and duration of inactivity) revealed that previous SA was independently associated with aBMD, at weight-bearing sites, and with cortical bone size of the tibia (cross sectional area (CSA), periosteal circumference (PC)). Previous SA explained 7.9% of the total variation in cortical CSA. Subjects, who ceased their sport activity for up to 6.5 years previously, still had greater cortical PC and CSA of the tibia, at the age of PBM, than ever inactive subjects.
In conclusion we demonstrate that SA during childhood and adolescence is associated with aBMD and bone geometry in currently physically inactive Swedish men at the age of PBM, suggesting that SA during growth confers positive effects on bone geometry even though SA is ceased.

***Disclosures: M. Nilsson**, None.*

SA542

See Friday Plenary number F542.

SA543

Path Analysis (Structural Equation Modeling) and Covariation of Bone Traits following Delayed Puberty. V. R. Yingling. Kinesiology and Anatomy and Cell Biology, Temple University, Philadelphia, PA, USA.

Functional interactions among morphological and tissue-quality bone traits are hypothesized to be part of a biological paradigm that results in multiple paths to achieve organ-level functionality. This focus on relationships between bone traits and not individual bone trait differences may elucidate new mechanisms of bone adaptation.

Therefore, the purpose was to illustrate that functional relationships between bone traits and body weight were altered by a delay in puberty using path analysis.
Twenty-three day old female rats were randomly assigned into a control group and experimental groups that received injections of Gonadotropin releasing hormone antagonists. Femora were embedded for histomorphometric analyses. A path model was constructed to test the hypothesis that variability in bone size (T.Ar/Length) and body size (Body Weight) is related to variability in relative cortical area (Ct.Ar/T.Ar) and % ash content. The model for the control groups (Figure 1) had excellent "goodness of fit" indicators. The chi-square was non significant (p = 0.493). The CFI (Comparative Fit Index) was 1.0; greater than 0.9 indicates a good fit. The RMSEA (Root Mean Square Error of Approximation) was 0.00; less than 0.05 indicates a good model fit. The model indicates that 56% of the variation of Ct.Ar/T.Ar is explained by Body Weight and bone size. Similar results were found in mice. Percent ash content was not affected by body weight or (Ct.Ar/T.Ar). In summary, control animals alter their relative cortical area by a direct relationship with body weight. A negative relationship was found between bone size (T.Ar/Length) and relative cortical area. Comparing these results to the model for the delayed puberty group (Figure 2) indicates that these relationships are no longer valid. Following a delay in pubertal timing only 9% of the variation in Ct.Ar/T.Ar was explained by body weight and bone size. To sum up, the path analysis suggests that (for these data sets) there is an effect of delayed puberty on the relationship between body weight, bone size and relative cortical area.

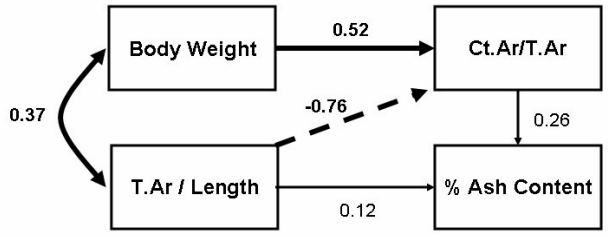


Figure 1:
Ct.Ar/T.Ar = 0.52BodyWeight – 0.76T.Ar/Length, **R^2=0.56**
Chi-square = 0.471, df=1, p-value=0.493
CFI=1.0, RMSEA=0.00

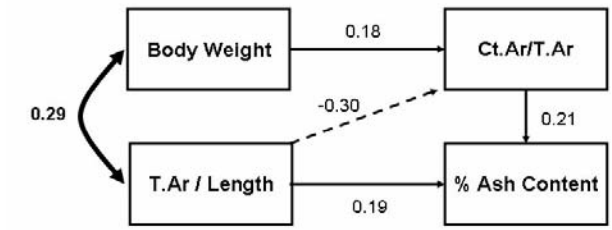


Figure 2:
Ct.Ar/T.Ar = 0.18BodyWeight – 0.30T.Ar/Length, **R^2=0.09**
Chi-square = 3.430, df=1, p-value=0.064
CFI=0.46, RMSEA=0.289

Disclosures: ***V.R. Yingling****, None.*

SA544

See Friday Plenary number F544.

SA545

Effect of Particle Size of Calcium Carbonate Supplement on Calcium Retention in Adolescent Girls. A. E. Elble*[1], K. M. Hill[2], C. Y. Park*[2], B. R. Martin[2], C. M. Weaver[2]. [1]Foods & Nutrition and Public Health, Purdue University, West Lafayette, IN, USA, [2]Foods & Nutrition, Purdue University, West Lafayette, IN, USA.

Previously we reported that substituting small for large (commercial standard) particle sized calcium (Ca) carbonate in diets of growing female rats resulted in higher calcium absorption and retention (JBMR 21: S184, Abst SA 368, 2006). Here we assessed the effect of large versus small calcium carbonate particle size and small particle Ca carbonate versus placebo on Ca retention in adolescent girls. Twenty-eight adolescent girls (13.1 ± 1.7y) participated in two 3-wk controlled feeding trials separated by a 1-wk washout period. During both sessions, the subjects consumed a diet identical in nutrient profile tailored to their individual energy needs containing 650 mg Ca/d. Using a cross-over design, one group (n=18) received an additional 650mg/d Ca via supplementation delivered twice a day in a 325mg Ca dose as either an average of 18 micron sized Ca carbonate (large) or 13 micron sized Ca carbonate (small). A second group (n=10) received the small particle sized Ca carbonate or placebo. All 24-h urine and feces were collected throughout the study and analyzed for calcium content by Inductively Coupled Plasma Spectrophotometry (ICP). The overall balance results (Balance = Ca intake - urine Ca - fecal Ca) indicated that the small particle size supplement resulted in greater calcium retention compared to placebo ($p<0.05$). However, there was no significant difference ($p = 0.39$) in Ca retention due to particle size of the supplement. Thus, the rat model did not predict the effect of particle size on Ca retention in humans. Unlike the rat study which started the treatment diet during weanling and continued throughout adolescence, the human subjects received the treatment during peak skeletal acquisition. Perhaps the overwhelming effects of growth during puberty overpowered the more subtle effect of Ca particle size treatment.

Disclosures: ***A.E. Elble****, Delavau 3.*
This study received funding from: Delavau.

SA546

See Friday Plenary number F546.

SA547

The Effects of Growth Hormone on Bone Micro-Architecture by *In Vivo* Micro-CT. E. Kristensen*[1], B. Hallgrímsson[2], D. W. Morck*[3], S. K. Boyd[1]. [1]Mechanical and Manufacturing Engineering, University of Calgary, Calgary, AB, Canada, [2]Cell Biology and Anatomy, University of Calgary, Calgary, AB, Canada, [3]Faculty of Veterinary Medicine, Faculty of Science, University of Calgary, Calgary, AB, Canada.

Children deficient in growth hormone (GH) are typically treated with injections of GH to attain normal body size. Dosage, duration of treatment, and age of onset of treatment all contribute to body shape and size. Untreated GH deficiency during puberty leads to low lumbar spine bone mineral density (BMD) when compared to age-matched healthy controls. Also, increasing the duration and decreasing the age of onset of treatment contribute to improved BMD [1]. BMD is the current gold-standard parameter to assess efficacy of GH treatment on bone. Presently, the effect of GH treatment on bone micro-architecture has not been investigated, as micro-architecture cannot be derived from BMD measurements.
The study's purpose was to perform a longitudinal assessment of the onset of GH treatment on bone micro-architecture using micro-computed tomography (micro-CT).
Ghrhr homozygous 'little' mice have reduced GH synthesis and release, although injection of exogenous GH can stimulate growth. Heterozygous mice have normal GH synthesis and release. A total of six experimental groups were studied. Two groups of homozygous mice received daily injections of GH starting at 21 days of age (early treatment group; ETG), or at 35 days of age (late treatment group; LTG). Two additional homozygous groups included vehicle controls (daily saline injections) and untreated controls. All injections ended at age 60 days. Two heterozygous groups were examined: a group receiving no treatment and a radiation control group. Micro-CT scans of the fourth lumbar vertebra were obtained for all groups (except radiation control) at age 21, 27, 35, 45, and 60 days (vivaCT 40, Scanco Medical AG). The radiation control group was scanned at days 21 and 60 only. Bone architectural parameters were calculated (IPL v. 4.29d, Scanco) and statistical tests determined treatment effects ($p<0.05$).
Relative to heterozygotes, there is a significant reduction in trabecular bone thickness in the untreated 'little' mice. There is no change in micro-architecture in the ETG as compared to the untreated homozygous mice. In the LTG relative to the untreated GH deficient mice, there is a trend towards increased bone quality, however these results are non-significant, likely due to small sample sizes (n=4 to n=8) at the current stage of study. Additional animals are being added to each group to increase statistical power. The data suggests support for the hypothesis that there is a benefit to late treatment, but no gains in bone quality when GH treatment is administered early.
[1] Mukherjee, A. et al. *Med Pediatr Oncol*, 2003. 41:235-42

Disclosures: ***E. Kristensen****, None.*

SA548

See Friday Plenary number F548.

SA549

Prevalence and Incidence of Viral Infections among Musculoskeletal Tissue Donors and First-Time Blood Donors. F. Yao*, M. Zheng, D. Wood*. Centre for Orthopaedic Research, University of Western Australia, Nedlands WA, Australia.

Musculoskeletal tissue is second only to blood as the most frequently transplanted human tissue, and there continues to be an enormous demand for these allografts throughout the world. Little information is known about the risks associated with musculoskeletal tissue donation.
Our objective was to define the prevalence and incidence of viral markers of human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), and human T-cell lymphotrophic virus (HTLV) in musculoskeletal tissue donors in Australia, and to compare the results with recently published data on rates of viral infection among tissue donors from Canada, Scotland, and the United States.
We studied blood serum samples from 12,415 consecutive musculoskeletal tissue donors from 3 large musculoskeletal tissue banks in Australia from 1993 through 2004. We defined prevalence as the number of donors with confirmed positive test results divided by the total number of donors tested. We estimated the incidence of new infections among donors by using a method similar to that of Zou and colleagues (1). We then compared the serologic data with those of first-time blood donors obtained during the same period.
The prevalence of viral markers in Australian musculoskeletal tissue donors was much higher than in Australian first time blood donors, by factors of about 10 for HIV, 3 for HBV, 2.5 for HCV, and 35 for HTLV ($P<0.05$). The prevalence of viral markers was greater in tissue donors than blood donors in all countries, except for anti-HIV antibody in Scottish tissue donors (Table 1). The prevalence of viral markers was higher in tissue donors in Australia than in Canada and Scotland. Estimated incidence rates of viral infections were also higher among tissue donors than first-time blood donors in Australia, Canada, and the United States.
We believe these findings have important implications. Monitoring viral prevalence and incidence among tissue donations is a vital tool for evaluating the safety of the tissue supply and provides important information with which to implement appropriate pubic health measures and policies.

[1] Zou S, Dodd RY, Stramer SL et al. Probability of viremia with HBV, HCV, HIV and HTLV among tissue donors in the United States. N Eng J Med. 2004; 351: 751-9.
[2] Zahariadis G, Plitt SS, O'Brien S, et al. Prevalence and estimated incidence of blood-borne viral pathogen infection in organ and tissue donors from Northern Alberta. Am J Transplant. 2007; 7: 226-34.
[3] Galea G, Dow BC. Comparison of prevalence rates of microbiological markers between bone/tissue donations and new blood donors in Scotland. Vox Sang. 2006; 91: 28-33.

Disclosures: ***F. Yao****, None.*

SA550

See Friday Plenary number F550.

SA551

Born Small Is Not Bad for Bone. Q. Wang[1], A. Iyytikainen[2], M. Alen[3], F. Tylavsky[4], E. Seeman[1], S. Cheng[2]. [1]ECE, Repatriation Hospital/Austin Health, The University of Melbourne, Heidelberg West, VIC 3081, Australia, [2]Health Sciences, The University of Jyvaskyla, Jyvaskyla, Finland, [3]The University of Oulu, Oulu, Finland, [4]Department of Preventive Medicine, The University of Tennessee Health Science Center, Knoxville, TN, USA.

Intrauterine growth is believed to be important in development of peak bone mass (PBM). We tested the hypothesis that born small adversely affects bone mineral content (BMC) at maturity. Crown-heel length (CHL) at birth and 1 year were documented from growth charts while BMC and areal bone mineral density (aBMD) of total body and sub-regions were assessed by dual-energy X-ray absorptiometry (GE Lunar) at 18.3 ± 1.0 yrs in 150 Finnish girls. Gestational age at birth ranged from 33 to 43 weeks (40 ± 1.5 weeks). Contrary to our hypothesis, BMC or aBMD of any site at 18 yrs was not predicted by CHL or birth weight (all NS). Growth rate in the first year of life did not track; 23% of girls increased their trait percentile and 23% decreased their trait percentile by > 1 quartile of their CHL despite no difference in their gestational age at birth (39.4 ± 1.2 vs. 40.4 ± 1.1 weeks, $p = 0.084$). Girls with catch-up growth were shorter at birth than those with slowdown grow (48.6 ± 1.0 vs. 50.9 ± 1.2, $p < 0.01$), but longer (77.1 ± 1.7 vs. 73.4 ± 1.4 cm) at 1yr and taller at 18 yrs (168.1 ± 4.5 vs. 163.7 ± 4.4, $p < 0.01$). Although being born smaller, BMC of total body and sub-regions at 18 yrs of age was 12.4% to.17.4% more in girls with catch-up growth than those with slowdown growth ($p < 0.01$). This difference in BMC at 18 yrs between the two groups was independent of CHL and weight at birth, but not independent of CHL and weight at 1yr or 18 yrs. Being born small is not a risk factor of suboptimal PBM, perhaps due to programming that facilitates expression of the phenotype during the first year of life.

Disclosures: ***Q. Wang****, None.*

SA552

See Friday Plenary number F552.

SA553

Relationship between Physical Fitness and Bone and Physical Activity and Calcium Retention in Adolescent Girls. K. M. Hill, A. E. Elble*, C. Y. Park*, B. R. Martin, S. L. Mobley*, C. M. Weaver. Foods and Nutrition, Purdue University, West Lafayette, IN, USA.

Adolescence is a crucial period for bone growth as half of the adult skeletal mass is accrued during this time. Physical activity has been associated with beneficial effects on bone mass in adolescents. However, the influence of physical activity on calcium (Ca) retention in adolescents is unknown. The purpose of this study was to determine the relationships between physical fitness scores and bone parameters and between Ca retention and physical activity (PA). Secondary data analysis from a 6-wk Ca metabolic balance study in adolescent girls (n = 48; ages 11-14 y) was performed. As a part of the metabolic study, subjects consumed a controlled diet and 24-hour urine and feces were collected. Ca retention was determined by dietary Ca intake minus urinary and fecal Ca. PA was assessed over four days during each 3-wk session by hip-worn omnidirectional accelerometers. Physical fitness was assessed by three fitness tests: push-ups, sit-ups, and sit-and-reach. Dual-energy x-ray absorptiometry (DXA), peripheral quantitative computed tomography (pQCT), and ultrasound (QUS) were used to determine bone parameters. Body composition was determined by DXA. Relationships among variables were analyzed by Pearson correlations; Paired t-tests were used to assess the influence of PA on Ca retention. Push-up fitness scores were positively associated with several bone parameters including radius total BMD and cortical BMD ($r = 0.37, 0.36$, respectively, $p < 0.01$) from pQCT and heel BMD ($r = 0.34$, $p < 0.05$) from QUS. Push-up fitness scores were also associated with percent fat mass ($r = -.37$, $p = 0.01$) and percent lean mass ($r = 0.38$, $p = 0.01$). PA assessed by accelerometry was not significantly related to Ca retention. PA may influence Ca retention if a broad range of PA were studied in contrast to our summer camp setting. Push-up fitness scores may best reflect habitual PA that influences bone mass at the forearm in adolescent girls.

Disclosures: ***K.M. Hill****, Delavau LLC 3.*
This study received funding from: Delavau LLC.

SA554

See Friday Plenary number F554.

SA555

The Effect of 12 Months Gymnastics Participation on Bone Mass Accrual in 4 to 7 Year Olds. M. C. Erlandson*, A. D. G. Baxter-Jones. College of Kinesiology, University of Saskatchewan, Saskatoon, SK, Canada.

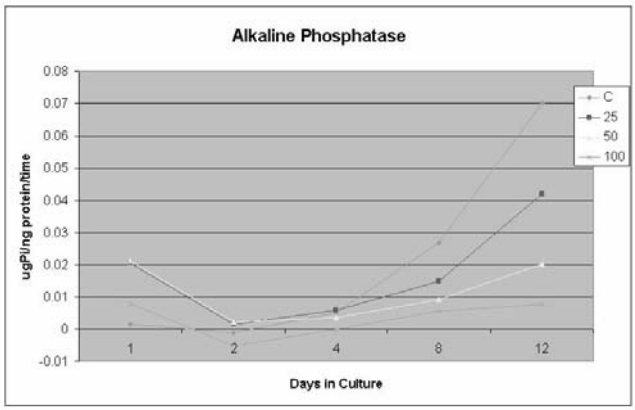
Studies of postpubertal gymnasts have constantly shown them to have greater bone mineral content (BMC) than age and maturity matched controls. However, it is unknown if this increased mineralization is the result of training or due to a genetic predisposition. The aim of this study was to assess the effect of 12 months gymnastics participation on BMC accrual in young prepubertal children. Gymnasts and controls were measured at baseline and after 12 months. Age, height, weight and dual-energy X-ray absorptiometry (DXA) of the whole body (WB), total hip (TH) and lumbar spine (LS) BMC were measured annually. Data were analyzed using analysis of variance (ANOVA) and covariance (ANCOVA; covariates, age, initial bone values, and changes in height and weight). Data are presented as mean ± SD. Three groups were identified: Gymnasts (Gym, n=54, age=5.6 ± 1.1), Gymnast who left the sport (Nongym, n=31, age=6.2 ± 1.2) and controls (Cont, n=75, age=5.9 ± 1.1). At study entry no significant differences ($p>0.05$) were found between the groups for age, height, weight or any BMC measures. At 12 months the mean adjusted BMC values for Gym, Nongym and Cont were 774.1 ± 8.7, 791.3 ± 9.9, 765.5 ± 7.2 g for WB, 9.5 ± 0.3, 9.7 ± 0.3, 9.2 ± 0.2 g for TH and 17.6 ± 0.2, 17.6 ± 0.3, 17.1 ± 0.2 g for LS (means adjusted for age, baseline BMC, change ht, change wt). No group differences were found at any site ($p>0.05$). The results from this study indicate that the effects of gymnastics training consistently reported in postpubertal adolescent samples were not present in this cohort at this young age, either a study entry or after 12 months of participation. To answer the question as to whether positive changes in bone mass accrual observed in adolescent gymnasts are due to prolonged training this cohort requires further follow-up measures spanning the pubertal period.

Disclosures: ***M.C. Erlandson****, None.*

SA556

Valproate: A Model for Adverse Psychoactive Drug Effects on Bone Mineral Density. B. L. Gracious[1], D. Heyer*[2], M. Parkhurst*[3], S. Messing*[4], J. Puzas[5]. [1]Psychiatry and Pediatrics, University of Rochester Medical Center, Rochester, NY, USA, [2]Neurology, University of Rochester Medical Center, Rochester, NY, USA, [3]Psychiatry, University of Rochester Medical Center, Rochester, NY, USA, [4]Biostatistics, University of Rochester Medical Center, Rochester, NY, USA, [5]Orthopedics, University of Rochester Medical Center, Rochester, NY, USA.

Purpose: To implement controlled translational experiments at cellular and human levels using valproic acid (VPA) as a model drug that may adversely affect BMD.
Aim I: Determine alkaline phosphatase (AP), Type I collagen, and osteocalcin production in osteoblasts exposed to VPA; Aim II: Compare BMD, bone biomarkers, and cortisol as potential mediators in teen girls with bipolar disorder treated with VPA.
Methods: AIM I: Primary rat osteoblasts (PROs) were exposed to control vs. VPA media, at human sub-, supra-, and therapeutic concentrations in culture. Protein was extracted across time points up to 18 days. Total protein and AP were measured via photospectroscopy, staining, and ELISA to examine changes in expression across time and conditions. AIM II: A cross-sectional matched study of teen girls taking VPA for Bipolar I disorder compared BMD via DXA and bone biomarkers with girls with BP I disorder not taking VPA and healthy controls.
Results: There is a differential effect of VPA on osteoblast AP production related to time and dose, with high therapeutic doses initially stimulating but then suppressing AP production. Interim analysis of DXA and biomarkers in bipolar teen girls exposed to VPA versus controls does not yet show separation between groups, however, salivary cortisol and BSAP trended higher in the VPA group.
Conclusions: VPA directly affects primary rat osteoblasts, altering alkaline phosphase production, which at higher therapeutic doses may result clinically in decreased bone formation. VPA-induced elevations of cortisol in humans may also play a role in adverse effects on BMD. There are likely multiple mechanisms by which VPA affects bone mineral metabolism. Clinical implications include that VPA dose and serum level necessary to treat patients may largely determine effect on BMD. Effects may also be different between cancellous and trabecular bone at different serum levels. Further basic and clinical prospective study of bone formation and resorption in response to VPA levels and sustained release dosing versus immediate release dosing is warranted.



***Disclosures:* B.L. Gracious**, *None.*
This study received funding from: NCRR 1 KL2 RR024136-1.

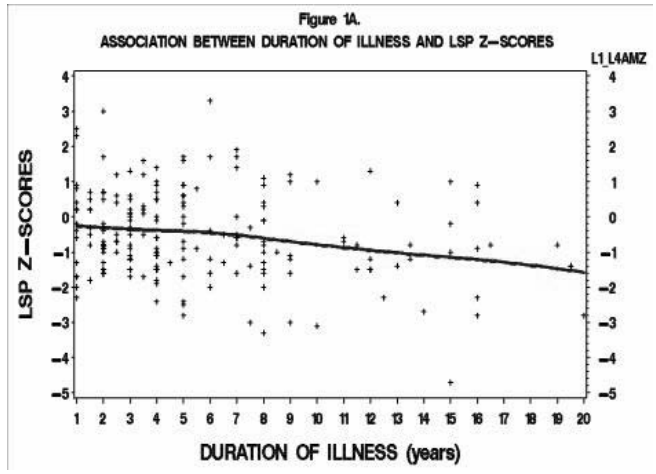
SA557

See Friday Plenary number F557.

SA558

BMD Changes Over Time Associated with Illness and Recovery in Young Women with Anorexia Nervosa. E. J. Waugh[1], B. Woodside*[2], P. Cote*[3], D. E. Beaton*[4], G. A. Hawker[1]. [1]Osteoporosis, Women's College Hospital, Toronto, ON, Canada, [2]Psychiatry, University Health Network, Toronto, ON, Canada, [3]Rehabilitation Solutions, University Health Network, Toronto, ON, Canada, [4]Mobility Program, St. Michael's Hospital, Toronto, ON, Canada.

We examined the effect of duration of illness and recovery of anorexia nervosa (AN) on BMD in a retrospective cohort study of patients aged 17-40 yrs who had received inpatient treatment for AN over a 12-yr time period. A detailed illness history was obtained by a Life History Calendar semi-structured interview. BMD at the lumbar spine L1-L4 (LSP), femoral neck (FN) and total body (TB) was measured by DXA. Low BMD was defined as a Z-score value ≤ -1.5 at one more sites.
We recruited 190 participants from two centers. At interview, mean age was 27.2 ± 5.6 yrs and mean BMI was 19.4 ± 3.4 (11.1 - 35.5) kg/m^2. Age at AN onset was 18.2 ± 4.2 (10-32) yrs, duration of illness was 5.9 ± 4.6 (1 - 25) yrs and lowest lifetime BMI was 13.9 ± 2.3 (7.2 - 18.0) kg/m^2. Eighty-three participants were recovered (achieved a BMI ≥ 18.5 kg/m^2 and resumed menstruation); duration of recovery was 4.8 ± 3.9 (0.8 - 26) yrs. Linear regression was used to estimate the rate of change in BMD Z-score values per year of illness and recovery, adjusted for weight.



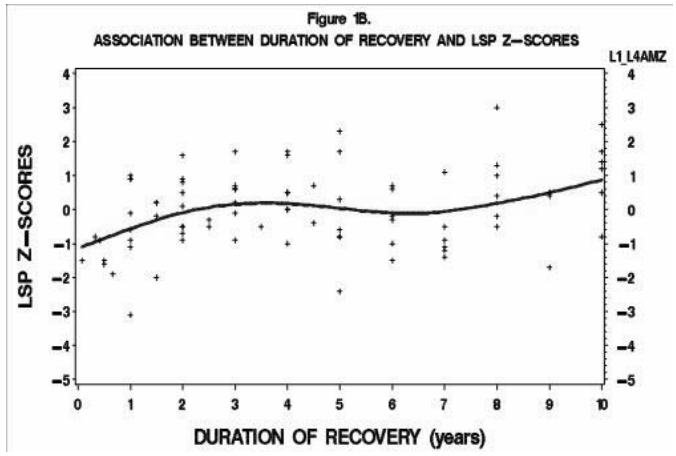


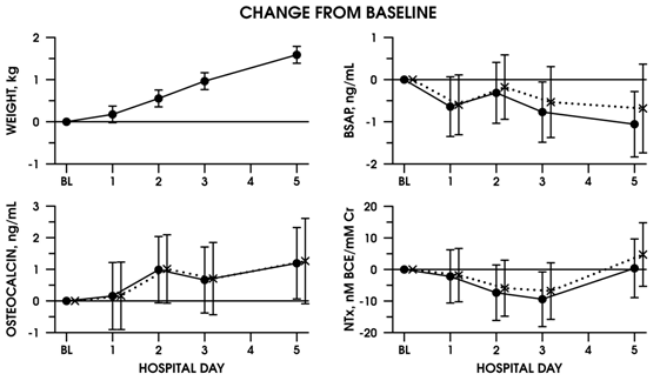
Figure 1 shows the relationships between durations of illness and recovery and LSP z-scores. Similar relationships were observed at the FN and TB (data not shown). A gradual and linear loss of BMD was associated with duration of illness (β = -0.05/yr, 95% CI -0.09, -0.009). The decline in LSP z-scores commenced immediately after onset of AN: 26.7% of participants ill for 1 yr had low BMD. In contrast, a rapid increase in BMD was associated with the first 3 yrs of recovery (β = 0.59/yr, 95% CI 0.23, 0.95) followed by stabilization of BMD after 3 yrs (β = 0.02/yr, 95% CI -0.7, 0.12). Low BMD was observed in 67% of those recovered for < 1 yr but only persisted in 25% of those recovered for 1 yr and 10% of those recovered for ≥ 2 yrs.

***Disclosures:* E.J. Waugh**, *None.*

SA559

The Effect of Bed Rest on Bone Turnover in Adolescents Hospitalized for Anorexia Nervosa. A. D. DiVasta*[1], H. A. Feldman*[2], A. Quach*[1], C. M. Gordon[1]. [1]Adolescent Medicine, Children's Hospital Boston, Boston, MA, USA, [2]Clinical Research Program, Children's Hospital Boston, Boston, MA, USA.

Bone loss is a well-established medical complication of anorexia nervosa (AN). At highest risk for skeletal insults may be those adolescents who become so malnourished that they require medical hospitalization to manage their illness and low weight. Current clinical standards often require bed rest for the duration of hospital stay. However, studies of healthy adults demonstrate that even short-term immobilization leads to disruptions in normal patterns of bone turnover. Our study aimed to determine the effect of short-term bed rest and relative immobilization on bone turnover in adolescents hospitalized for AN.
In this observational study, 28 female adolescents (mean age=16.7 ± 2.3 y) were prospectively enrolled upon hospitalization. Eligible patients met DSM-IV diagnostic criteria for AN, and had no other co-morbid conditions or medication use known to affect bone health. As per standard care, all patients were placed on bed rest and graded nutritional therapy. Weight was measured daily, in a standard fashion. Markers of bone formation [bone-specific alkaline phosphatase (BSAP) and osteocalcin (OC)] and bone resorption [urinary N-telopeptides (NTx)] were measured in a fasting state at baseline and days 1, 2, 3, and 5 of hospitalization. Results are presented as adjusted trend estimates from repeated-measures regression analysis.
During the 5 days of hospitalization, mean weight increased by 0.33 kg/day ($p<0.001$). BSAP declined by 0.18 ± 0.07 ng/mL/day (p=0.01). Serum OC, interestingly, increased by 0.24 ± 0.1 ng/mL/day (p=0.02). Urine NTx reached a nadir on day 3, declining -7.0 ± 2.7 nM BCE/mM Cr (p=0.01), but then returned to baseline levels by day 5 ($p>0.05$; Figure 1). After controlling for weight gain, as well as age, baseline percentage of ideal weight, duration of amenorrhea, hours of weekly exercise, and family history of osteoporosis, the effect of time on BSAP was no longer significant (p=0.24).



Limitation of physical activity during hospitalization for patients with AN leads to a suppression of both bone formation and resorption, and a potential imbalance of bone turnover. Future interventional studies involving mechanical stimulation and/or weight-bearing activity are needed to determine whether medical protocols prescribing strict bed rest are appropriate.

***Disclosures:* A.D. DiVasta**, *None.*

SA560

The Effect of Vitamin D_2 and Vitamin D_3 on Intestinal Calcium Absorption in Nigerian Children with Rickets. T. D. Thacher[1], M. O. Obadofin*[2], K. O'Brien[3], S. A. Abrams*[4]. [1]Family Medicine, Mayo Clinic, Rochester, MN, USA, [2]Family Medicine, Jos University Teaching Hospital, Jos, Nigeria, [3]Division of Nutritional Sciences, Cornell University, Ithaca, NY, USA, [4]USDA/ARS Children's Nutrition Research Center, Baylor College of Medicine, Houston, TX, USA.

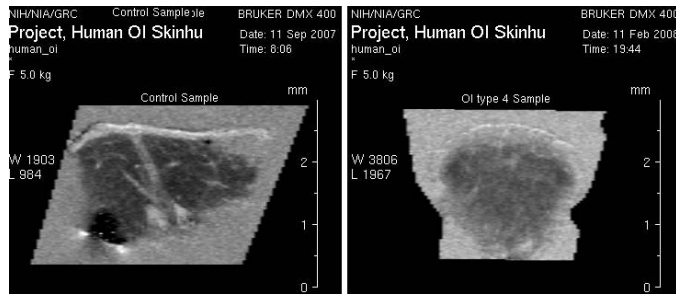
Children with nutritional rickets due to calcium deficiency have high serum $1,25(OH)_2D$ values. We examined if additional vitamin D increased calcium absorption.
We randomly assigned 17 Nigerian children with rickets, ages 2-10 years, to either vitamin D_3 (cholecalciferol; n=8) or vitamin D_2 (ergocalciferol; n=9) 1.25 mg given orally. Using stable isotope methods, fractional calcium absorption was determined at baseline and 3 days after vitamin D administration.
Mean daily dietary calcium intakes were 182±73 mg, and mean baseline 25(OH)D concentrations were 20 ng/mL (range 5-31 ng/mL). Three days after oral vitamin D, the increase in serum vitamin D_3 (52±22 ng/mL) in the cholecalciferol group and vitamin D_2 (48±18 ng/mL) in the ergocalciferol group demonstrated equivalent drug absorption. The increase in 25(OH)D was equivalent with cholecalciferol (29±10 ng/mL) and ergocalciferol (29±17 ng/mL). Mean $1,25(OH)_2D$ values increased from 143±76 pg/mL to 243±102 pg/mL (P=0.001), and the increase in $1,25(OH)_2D$ did not differ between ergocalciferol and cholecalciferol (107±110 and 91±102 ng/mL, respectively). The rise in $1,25(OH)_2D$ was explained almost entirely by the baseline 25(OH)D concentration ($r^2=0.72$; $P<0.001$). The increase in serum calcium was similar with cholecalciferol (0.29±0.34 mg/dL) and ergocalciferol (0.41±0.51 gm/dL; P=0.57) and significantly greater than baseline values (P=0.004). 24-hour urinary calcium excretion did not differ before (1.4±1.9 mg) or after (1.4±1.7 mg) vitamin D administration. Mean fractional calcium absorption did not differ before (52.6±21.4%) or after (53.2±23.5%) vitamin D administration, and there was no significant difference between the effect of ergocalciferol and cholecalciferol on calcium absorption. Fractional absorption of calcium was not closely related to concentrations of 25(OH)D (r=0.01, P=0.93) or $1,25(OH)_2D$ (r=0.21, P=0.24). The effect of vitamin D on calcium absorption did not vary with baseline 25(OH)D values or with the absolute increase in 25(OH)D or $1,25(OH)_2D$ values.
Despite the marked rise in $1,25(OH)_2D$ in response to oral vitamin D, consistent with vitamin D inadequacy, no augmentation of fractional absorption of calcium absorption occurred. Ergocalciferol and cholecalciferol appear to be bioequivalent.

***Disclosures:* T.D. Thacher**, *None.*

SA561

Identification of Skin Abnormalities in Osteogenesis Imperfecta Patients by Magnetic Resonance Imaging-A Pilot Study. C. L. Raggio*[1], K. W. Fishbein*[2], E. M. Carter*[3], M. Kim*[4], N. Pleshko[5], R. G. Spencer*[6]. [1]Orthopaedic Surgery, Hospital for Special Surgery, New York, NY, USA, [2]National Institutes on Aging, National Institutes of Health, Baltimore, MD, USA, [3]Kathryn O. and Alan C. Greenberg Center for Skeletal Dysplasias, Hospital for Special Surgery, New York, NY, USA, [4]Mineralized Tissue Laboratory, Hospital for Special Surgery, New York, NY, USA, [5]Biomechanics, Exponent, Philadelphia, PA, USA, [6]Nuclear Magnetic Resonance Unit, National Institute on Aging, National Institutes of Health, Baltimore, MD, USA.

Osteogenesis imperfecta (OI) is a genetic disorder characterized by bone fragility and frequent fractures. Diagnosis is based on clinical and radiological criteria, which can be nonspecific in mild-to-moderate cases, and increasingly by genetic testing, which is time-consuming. We tested the hypothesis that magnetic resonance imaging (MRI) can detect skin abnormalities that correlate with OI genotype and phenotype. Our primary research objectives were to determine: 1. whether MRI can differentiate between skin from OI and control subjects; 2. whether nondestructive MRI analysis is supported by invasive but highly specific Fourier transform infrared spectroscopic imaging (FT-IRIS); and 3. whether there is a relationship between type I collagen genotype, skeletal phenotype, and skin phenotype across patients of all ages. MRI analysis of 3-mm full-thickness forearm skin biopsies from OI (n=6) and non-OI control (n=2) subjects was performed, followed by FT-IRIS. MRI parameters, including T1, T2, and magnetization transfer (MT), were compared to FT-IRIS parameters characterizing dermal collagen. Initial findings showed clear differences in both MRI and FT-IRIS parameters between patients with OI and controls. Findings within the OI group correlated with the severity of clinical phenotype; epidermal and dermal layers were thinner in OI patients compared to controls (See attached T2 image) with the degree of thinning correlating with the severity of OI phenotype. MRI revealed fat deposits within the dermis of OI skin only. FT-IRIS revealed differences in collagen orientation in the dermis of OI skin compared to controls. We conclude that MRI is sensitive to presence and severity of OI in human skin, as confirmed by FT-IRIS analysis. This supports the potential for developing an MRI approach for rapid non-invasive diagnosis of children with OI.



***Disclosures:* C.L. Raggio**, *None.*

SA562

Autosomal Recessive Hypophosphatasia Manifesting *in Utero* with Long Bone Deformity but Showing Spontaneous Postnatal Improvement. D. A. Stevenson*[1], J. C. Carey*[1], S. P. Coburn*[2], K. L. Ericson*[2], J. L. B. Byrne*[3], S. Mumm[4], M. P. Whyte[4]. [1]Division of Medical Genetics, Dept Pediatr, Univ Utah; Shriners Hospt Children, Salt Lake City, UT, USA, [2]Dept Chemistry, Indiana Univ-Purdue Univ, Fort Wayne, IN, USA, [3]Div Maternal-Fetal Medicine, Dept ObGyn, Univ Utah, Salt Lake City, UT, USA, [4]Washington Univ Schl Med; Shriners Hospt Children, St. Louis, MO, USA.

Hypophosphatasia (HPP) is the heritable metabolic disorder of the skeleton and dentition featuring highly variable expressivity conditioned primarily by gene dosage effect and the variety of loss-of-function mutations in the tissue nonspecific alkaline phosphatase gene *(TNSALP)*. Extracellular accumulation of the TNSALP substrate inorganic pyrophosphate, an inhibitor of skeletal mineralization, accounts for the accompanying rickets or osteomalacia. Four principal forms have been characterized based on age at diagnosis of underlying skeletal disease: perinatal, infantile, childhood, and adult

HPP. Patient age when skeletal problems first manifest generally predicts the clinical course, with autosomal recessive perinatal HPP causing bone disease *in utero* that is obvious at birth and typically lethal soon after. Milder forms may be due to autosomal dominant or autosomal recessive inheritance.

We describe a boy with HPP detected prenatally by sonography showing long bone deformity (Figure) which spontaneously improved *in utero* and after birth. His older brother has the childhood form of HPP without clinical findings until after infancy. Both boys are compound heterozygotes for the same *TNSALP* missense mutations (c.526G>A, p.Ala176Thr in exon 6, and c.814C>T, p.Arg272Cys in exon 8), documenting autosomal recessive inheritance for their HPP. The parents are carriers for HPP, but are clinically unaffected (Table).

Our findings show that prenatal detection of HPP by sonography because of bowing of long bones caused by autosomal recessive inheritance does not necessarily predict lethality, but can represent variable expressivity or the effects of modifiers on the *TNSALP* defects.

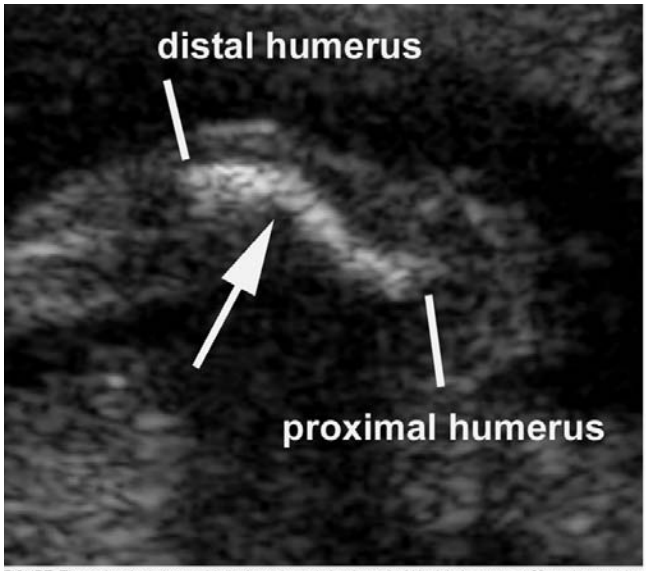


FIGURE: The patient had sharp, mid-diaphyseal angulation (arrow) of his right humerus at 20 weeks gestation.

TABLE Biochemical Studies Indicating Hypophosphatasia

Test	Patient (Sib #1)	Father	Mother	Sister (Sib #2)	Brother (Sib #3)		Normal Range*	
Age (years)**	3	29	28	4	2.5	6	Pediatric	Adult
Serum								
ALP (IU/L)	47	55	32	308	36	63	133-347	30-114
Bone Specific ALP† (IU/L)	29	21	11	144	30	36	47-181	3-38
Plasma								
PLP (nmol/L)	3,505	191	258	117	1,332	4,034	5-107	

* Normal ranges (± 2 SD mean) for were constructed from data from fasting blood specimens obtained from 20 children ages 4.6 – 12.9 years with ad libitum diets and assayed at Shriners Hospital for Children, St. Louis, MO, USA in 1997. ALP = alkaline phosphatase. The range for plasma pyridoxal-5′-phosphate (PLP) concentration is appropriate for both children and adults. (Whyte et al, JBMR 18:624, 2003)

** Age when samples for biochemical studies were obtained.

† Metra Biosystems, Inc., Mountain View, CA, U.S.A.

***Disclosures: M.P. Whyte**, None.*

SA563

See Friday Plenary number F563.

SA564

Prevalence of Transient Hyperphosphatasemia among Healthy Infants and Toddlers. S. Y. Huh*, H. A. Feldman*, C. M. Gordon. Children's Hospital, Boston, MA, USA.

Transient hyperphosphatasemia (TH) is a condition characterized by a temporary elevation of serum alkaline phosphatase in the absence of bone or liver disease. The epidemiology and etiology of TH are not well-understood. Our study goal was to describe the prevalence and clinical characteristics of TH in a cohort of healthy infants and toddlers. We performed a secondary data analysis of 364 children, aged 8 to 24 months, enrolled in a study examining the epidemiology of vitamin D deficiency. After obtaining parental consent, children were enrolled at well-child visits conducted from October 2005 to June 2007 in an urban primary care pediatric clinic. Children with a chronic disease or using medications known to affect bone metabolism were excluded. At enrollment, we collected data regarding child age, gender, height, and weight; maternal race/ethnicity; and season (fall/winter, October to March, or spring/summer, April to September). We measured serum levels of alkaline phosphatase (AP), 25-hydroxyvitamin D [25(OH)D], parathyroid hormone, calcium, and magnesium. We defined TH as an AP > 1000 U/L at enrollment. We examined simple associations and used multiple logistic regression to evaluate the association between clinical characteristics and TH.

Nine of 364 children (2.5%) had an AP > 1000 U/L (mean 2165 U/L, range 1006 to 4293 U/L). Twenty-four children had a high AP that did not meet the definition for TH (mean 546 U/L, range 423 to 835 U/L). Three hundred thirty-two children had a normal AP (mean 261 U/L, range 100 to 400 U/L). Among the 9 children with TH, mean age was 11.8 months (range 9.0 to 19.0 months) and 5 children (56%) had mothers of black race. Fifty-six percent of children with TH were enrolled during the winter, compared with 50% of children with a normal AP. Compared to the 332 children with a normal AP, children with TH had similar mean serum levels of 25(OH)D (32.3 vs. 34.9 ng/mL), PTH (30.0 vs. 27.6 pg/mL), calcium (10.6 vs. 10.4 mg/dL), and magnesium (2.3 vs. 2.3 mg/dL); mean weight-for-length Z-score (0.23 vs. 0.31 SD) and length-for-age Z-score (-0.09 vs -0.06 SD) were also similar. Child age, gender, weight-for-length Z-score, length-for-age Z-score, and serum 25(OH)D levels were not associated with TH in a multivariable model. In conclusion, the 2.5% prevalence found within this study suggests that TH is not a rare condition among healthy infants and toddlers. In this small sample, TH was not associated with anthropometric measures, vitamin D deficiency, or biomarkers of bone turnover. Recognition of this benign condition is important to avoid unnecessary investigations.

***Disclosures: C.M. Gordon**, None.*
This study received funding from: Allen Foundation Inc.

SA565

Pediatric Crohn Disease Is Associated with Negative Calcium Balance and Increased Renal Losses of Calcium. F. A. Sylvester[1], M. Lincoln*[1], L. T. Fourman*[2], K. O. O'Brien*[2]. [1]Pediatric Gastroenterology, Connecticut Children's Medical Center, Hartford, CT, USA, [2]Division of Nutr Sciences, Cornell University, Ithaca, NY, USA.

Pediatric Crohn disease (CD) is associated with significant deficits in bone mass, but the responsible mechanisms are not clear. The purpose of this study was to examine calcium balance in children with CD in remission using stable isotopes of calcium. We excluded subjects exposed to glucocorticoids in the previous 3 months. We enrolled 9 subjects (mean 12.9 ± 2.6 [9.1-15.4] y, 7 M). We measured BMD by DXA (GE Lunar Prodigy Advanced). Subjects received 972 ± 47 μg/kg ^{42}Ca IV over 5 min and 3777 ± 117 μg/kg ^{44}Ca in milk as part of a meal. We recorded their food intake for subsequent dietary analysis. During a 1-day inpatient stay, we collected timed samples of blood, a 24-h urine collection, and all stools. As outpatients, subjects collected 3 spot urine samples/day and all stools for 5 days. Calcium was extracted from each sample for analysis by mass spectrometry (Thermoquest Triton TI Magnetic Sector Thermal Ionization Mass Spectrometer) and atomic absorption spectrometry. We determined fractional absorption of calcium and endogenous fecal losses. In addition, we performed sum-of-exponential and multi-compartmental modeling of the time-dependent disappearance of intravenous tracer from plasma with the Simulation, Analysis, and Modeling computer program. Data were compared to published normative age- and sex-matched data. We used two-tailed paired t-tests to determine significance between CD and healthy subjects, and two-tailed unpaired t-tests when data was not available to match each subject to an appropriate healthy counterpart. We compared the two groups in terms of Ca intake, intestinal absorption, bone deposition, bone resorption, urinary calcium excretion, endogenous fecal excretion, and calcium balance. Mean BMI Z-score was -0.61 ± 0.79 kg/m^2. Lumbar spine BMD Z-score was <-1 in 4/9 subjects with CD (mean -0.70 ± 1). Average calcium intake was 1257 ± 446 mg/d. Mean bone Ca deposition was lower than expected in relation to normative data in healthy children (1.98 ± 0.34 g/day; p = 0.013), whereas fractional absorption of calcium was normal. Calcium balance was significantly lower in combined Tanner stages 3 and 4 subjects with CD (37 ± 25 mg/day; p= 0.029). Urinary calcium excretion exceeded values in healthy counterparts by 104 ± 127 mg/day (p = 0.040). Fecal excretion was not significantly different than in controls. In summary, children with CD in remission are in a state of negative calcium balance, especially in late puberty, characterized by excessive urinary losses of calcium. Based on these data, we predict an annual loss of 1.6% of skeletal calcium, which may affect the achievement of peak bone mass.

***Disclosures: F.A. Sylvester**, None.*

SA566

c.1250A>G, p.N417S is a Common American *TNSALP* Mutation Involved in all Clinical Forms of Hypophosphatasia (HPP), Including Pseudo-HPP.
D. Wenkert[1], W. H. McAlister[2], S. Mumm[2], M. P. Whyte[2]. [1]Shriners Hospt for Children, St. Louis, MO, USA, [2]Washington Univ Schl of Medicine, St. Louis, MO, USA.

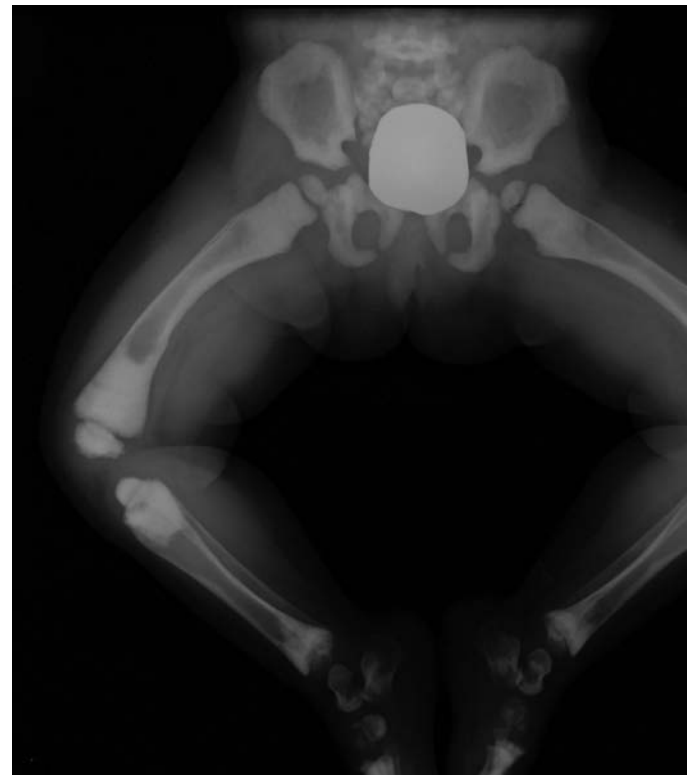
Hypophosphatasia (HPP) is an inborn error of metabolism caused by loss-of-function mutation(s) in the gene encoding the tissue nonspecific isoenzyme of alkaline phosphatase (*TNSALP*). HPP is highly variable in severity, ranging from tooth manifestations only, to death in utero from profound skeletal hypomineralization. The 7 clinical forms are perinatal, infantile (lethal in 50% of patients), childhood (rickets, +/- short stature, and delayed fracture healing), adult (fractures from osteomalacia), odonto-HPP (early loss of 1° dentition), " benign prenatal" (in utero bowing with improvement ex utero), and pseudo-HPP (infantile HPP phenotype, but normal serum alkaline phosphatase (ALP) activity in clinical laboratories).
We describe 19 individuals with a p.N417S mutation near the active site of TNSALP. Of the ~170 patients with HPP followed at our research center, 12 carried this c.1250A>G mutation (representing 7 families) and were studied with fasting serum, 24 hour urine collections, and radiologic studies. Serum and DNA were obtained from 7 additional unrelated patients. None was receiving multivitamins. All manifested TNSALP substrate accumulation. The average pyridoxal 5'-phosphate (PLP) and inorganic pyrophosphate (PPi) accumulation in compound heterozygotes was 3x greater than that in individuals with a single *TNSALP* c.1250A>G allele.
5 patients with c.1250A>G were compound heterozygotes, and presented with benign prenatal, infantile, childhood, and pseudo-HPP. Perinatal HPP in a compound heterozygote has been previously reported for the c.1250A>G *TNSALP* mutation. The 14 individuals who carry c.1250A>G as a single mutation of *TNSALP* represented carriers (6), odonto (4), mild childhood (1), adult (2), and benign prenatal (1). Thus, the c.1250A>G is a common, "mild," *TNSALP* mutation that may be susceptible to other genetic or environmental factors, and occurs in all forms of HPP, including pseudo-HPP.

***Disclosures:* *M.P. Whyte**, None.*

SA567

Dysosteosclerosis in a 2-Year-Old Girl: Investigation of a Rare, Sclerosing Bone Disorder.
D. Wenkert[1], W. H. McAlister*[2], S. Mumm[2], M. P. Whyte[1]. [1]Shriners Hospt Children, St. Louis, MO, USA, [2]Wash U Sch Med, St. Louis, MO, USA.

Dysosteosclerosis (OMIM % 224300) is an extremely rare, sclerosing bone disorder. Pts typically acquire short stature, optic atrophy, and blindness. Some develop cranial nerve palsies, developmental or dental problems, and frequent fractures. Bone histology suggests disrupted osteoclast function.
We investigated a 23 mo girl of Turkish descent whose episodic joint pain prompted a skeletal survey at age 7 mos. She was the 3rd child of unrelated parents in their 30's, and was 6-lb, 19", and 38-wks gestation at birth. At age 5 mo, CT for rapidly increasing head size showed prominent ventricles, sulci, and subarachnoid spaces. Tooth eruption and development were normal. She had recurrent ENT and skin infections. Between our evaluations at ages 11 and 23 mos, weight was ~30%, but length fell to -2.4 SD, and head circumference was +4 SD with frontal bossing, flattened nasal bridge, low-set ears, white sclera, 9 normal teeth, genu valgum, and normal joints.
Family history was negative for bone disease. Her mother, 5'1", had cholesteatomas with residual deafness. Her father was healthy. Their radiographs were unremarkable.
Pt x-rays showed progressive metaphyseal widening and sclerosis of all long bones, medial clavicular expansion, orbital and facial sclerosis, basilar thickening, "bone-in-bone" in the pelvis, 10 rib and one femur fracture, flattened and beaked vertebrae in keeping with dysosteosclerosis (Fig). With generous dietary calcium, she had slight hypercalcemia, intermittent hypercalciuria, and normal serum PTH and phosphorus. Alkaline phosphatase and osteocalcin seemed low. Heavy metal screening was negative. The significant elevations in serum LDH or the brain isoform of creatine kinase, seen in osteopetrosis (OPT), were absent. Hemogram suggested no bone marrow suppression. Commercial mutation analysis of the chloride channel 7 (*CLCN7*), T-cell immune regulator 1 (*TCIRG1*), and OPT-associated transmembrane protein 1 (*OSTM1*) genes revealed a heterozygous transition in *CLCN7* (T181M) thought not to be pathogenic because this amino acid is not conserved across species, and occured in her father. We showed no defects in the RANK (exon 1) or OPG genes, but an unreported heterozygous change in *TNFSF11* (RANKL, c.107C>G, p.P36R).
Our patient's findings suggest that dysosteosclerosis is a form of OPT, but without the serologic hallmarks or mutations in established OPT genes.



***Disclosures:* *M.P. Whyte**, None.*

SA568

First Clinical Application of Electric Stimulation on Human Distracted Bone.
Y. Nabil*[1], H. Selim*[2], O. Hegge*[3], M. D'Angelo[4], A. A. Selim*[5]. [1]Dental Surgery Department, Military Hospital, Cairo, Egypt, [2]Maxillary Oral Surgery, Ain Shams University Dental School, Cairo, Egypt, [3]Rehabilitation and Rheumathology Deaprtment, Ain Shams University School of Medicine, Cairo, Egypt, [4]Anatomy and Cell Biology, Philadelphia College of Osteopathic Medicine, Philadelphia, PA, USA, [5]College of Science, Biomedical research group, KFUPM, Dhahran, Saudi Arabia.

Skeletal tissue is subjected to several biological and physiological forces that modulate its regenerative responses. Management of skeletal deformities in the maxillofacial region has presented a challenge to clinicians. Successful animal model applications of distraction osteogenesis (DO), also refer to as osteodistraction, in the maxillofacial complex has been extensively reported. DO is a surgical technique that uses the body's own repairing mechanisms as allies for optimal tissue reconstruction and this method has joined the conventional techniques for comprehensive treatment of patients with skeletal insufficiencies. Distraction osteogenesis is known to produce mechanical strains that are transformed by bone cells into electrical signals. In accordance with that, several animal studies suggested that the application of electric current may stimulate bone cells to form new bone. In this study, we report the first clinical application of electric stimulation on human distracted bone. Six adult patients underwent segment transfer distraction osteogenesis of mandibular defects combined with the application of direct electric current. We hypothesized that osteodistraction accompanied with electrical stimulation, will make higher speed feasible without compromising bone quality. Direct current of 10 µA was started at 7 days postoperatively and continued throughout activation and consolidation periods with a rate of distraction at 2mm per day. The distracted and control non-distracted sides were compared during latency, activation and consolidation periods at 1, 3, 6 and 12 month post operatively. Clinical examination, ultrasonography, digital plain radiographs, bone densitometry and computed tomography demonstrated marked increase in bone density of the distracted bone during the distraction process, at the end of consolidation period and surprisingly the distracted bone demonstrated higher densities compared to control. In conclusion, electric stimulation was an effective method to enhance bone formation in mandibular osteodistraction cases. These data suggests that electric stimulation during osteodistraction may be a good modality to shorten the time needed for distraction osteogenesis and to insure high bone quality during distraction process.

***Disclosures:* *A.A. Selim**, None.*

SA569

See Friday Plenary number F569.

SA570

Alfacalcidol Prevents Aromatase Inhibitor (Letrozole) Induced Bone Mineral Loss in Young Growing Female Rats. M. H. Idris*, X. Q. Liu, J. K. Yeh. Metabolism Lab., Winthrop Univeristy Hospital, Mineola, NY, USA.

Long-term aromatase inhibitors use causes bone loss and increase fracture risk secondary to induced estrogen deficiency. We postulated that alfacalcidol (vitamin D3 analog) could prevent the letrozole induced bone mineral loss. Fifty intact 1 month old female rats were randomly divided into five groups with 10 rat each; basal group (B) was sacrificed at start of the experiment; age matched control group (C); letrozole group (L) oral administration of 2 mg/kg per day; alfacalcidol group (A): oral administration of 0.1 ug/kg per day; group L+A: letrozole 2 mg/kg + Alfacalcidol 0.1 ug/kg per day for a period of 8 weeks. When animal sacrificed, serum estrone (E1), estradiol (E2), testosterone (T), LH, FSH, IGF-1 were measured and femoral bone mineral density were scanned by DEXA. Data was analyzed by One-Way ANOVA with group comparison. Eight weeks administration of Letrozole resulted in a significant increase in body weight, tibial and femoral bone length and a significant decrease in the ovary+utrus horn weight as compared to the non-treated groups with or without alfacalcidol. Letrozole administration increased serum T, LH, FSH, IGF-1 and suppressed E1 and E2 significantly. None of those parameters were affected by Alfacalcidol treatment. The result of DEXA shows that Letrozole increased femoral bone area (1.8±0.09cm^2 vs C; 1.64±0.07cm^2 (mean±S.D.), P<0.05) without a significant effect on the bone mineral content (0.39±0.02g vs C; 0.38±0.02g). Therefore, the BMD (217.8±4.4mg/cm^2) was significantly lower than that of the control group (229.8±4.9mg/cm^2, P<0.05). Alfacalcidol increased the bone area (1.78±0.07cm^2, P<0.05), bone mineral content (0.44±0.03g, P<0.05) and BMD (249.8±5.9mg/cm^2, P<0.05) as compared to the C group. L+A combine intervention resulted in a significant increase in the bone area (1.91±0.09cm^2, P<0.05 vs C, L and A groups), bone mineral content (0.46±0.03g, P<0.05 vs C and L groups) and BMD (236.8±8.0mg/cm^2, P<0.05 vs C and L groups). This study demonstrates that Letrozole treatment to the young growing female rats enhances body weight and bone elongation due to elevation of both T and IGF-I with the suppression of E1 and E2. However, the bone density decreased significantly. We believe that Letrozole induced reduction in BMD was secondary to increase bone volume without corresponding increase in mineral deposition. Increased mineral deposition to the growing bone by Alfacalcidol demonstrates that it has theraputical value in preventing Letrozole induced bone osteopenia.

Disclosures: ***M.H. Idris****, None.*

SA571

The Effects of Long-term Administration of High-dose Growth Hormone on Body Composition and Bone Mineralization. L. K. Branski*[1], D. N. Herndon*[2], G. Kulp*[2], M. G. Jeschke*[2], W. B. Norbury*[2], K. E. Naylor[3], R. Eastell[3], G. L. Klein[4]. [1]Surgery, Shriners Burns Hospital and University of Texas Medical Branch, Galveston, TX, USA, [2]Surgery, University of Texas Medical Branch and Shriners Burns Hospital, Galveston, TX, USA, [3]Academic Unit of Bone Metabolism, University of Sheffield, Sheffield, United Kingdom, [4]Pediatrics, University of Texas Medical Branch and Shriners Burns Hospital, Galveston, TX, USA.

Recovery from massive burn injury is marked by persisting catabolism for up to one year. Treatment with recombinant human growth hormone (rhGH) is reported safe and effective in promoting an anabolic response following burn. The aim of this study was to assess the effects of long-term (one year) treatment with high doses of rhGH on body composition and bone metabolism. 161 pediatric patients with burns >40% total body surface area were studied from 2000-2006. They were prospectively randomized to receive either placebo (control group, n=93) rhGH at a daily dose of 0.1 mg/kg (0.1 group, n=46), or 0.2 mg/kg (0.2 group, n=22) for one year after discharge. Patients were followed from discharge at 6,9,12, and 24 mo post-burn (intent to treat study design). Weight, lean body mass (LBM), total body bone mineral content (BMC) and % total body fat (FAT) were measured at each time point. IGF-1, IGFBP3, PTH, osteocalcin, and serum total calcium were also measured at admission. Urinary NTx was measured at 12 mo. Statistical analysis was performed using Tukey's t-test or repeated measures ANOVA followed by Bonferroni's t-test. In each case %change of each parameter from discharge was compared vs controls. Significance was accepted at p<0.05. LBM increased at 9,12, and 24 mo in the 0.1 group (p<0.04) and in the 0.2 group beginning at 6 mo (p<0.02). FAT decrease was seen in the 0.1 group (6,9,12,24 mo, p<0.03) and in the 0.2 group (p<0.04). IGF-1 levels were elevated in both treatment groups compared to control (p<0.05). BMC fell in the 0.2 group at 9 and 12 mo (p<0.02) with elevated osteocalcin (p <0.002) and decreased PTH (p<0.01) at 9 and 12 mo postburn. Urine N-Tx trended toward increase at 12 mo (p=0.06). We conclude that high dose rhGH increases LBM in burned children but with an accompanying decrease in total body BMC and increased bone resorption in the 0.2 mg/kg/d dose at 12 mo post-burn. Data are insufficient at present to determine whether BMC recovers at 24 mo but if as in the GH-associated bone resorption of GH-deficient children at 12 mo it takes another year of administration to show an increase in BMC, cost vs benefit of treatment must be determined to see if any additional therapy is worthwhile.

Disclosures: ***G.L. Klein****, None.*
This study received funding from: National Institutes of Health.

SA572

See Friday Plenary number F572

SU001

Continuous Local Infusion of Alendronate Prevents Osteopenia of the Lengthened Segment During Distraction Osteogenesis. A. Abbaspour[1], T. Baghdadi*[1], R. Espandar*[1], S. Saberi*[1], P. Heidari*[1], S. Takata[2], N. Yasui[2]. [1]Orthopedics, Tehran University/ medical science, Tehran, Iran, Islamic Republic of, [2]Orthopedics, Health Bioscience, Tokushima, Japan.

Distraction osteogenesis is a well-established method for bone lengthening with widespread clinical application. However to achieve extensive bone regeneration, the external fixater must be applied for a long period, which may result stress-shielding osteopenia. In this study we performed the ability of continuous local injection of alendronate to the lengthened segment of distraction osteogenesis for prevention of osteopenia.
Seventy-two male rabbits had subperiosteal osteotomy of the left tibia and an external fixator was applied anteromedially. There was a lag phase for one week, a distraction phase for two weeks, and a consolidation phase for five weeks. At the beginning of consolidation phase, alendronate was injected continuously at a rate of 7.14 μg/kg/day into the lengthened segment through a needle and catheter connected to a subcutaneously implanted osmotic pump. Control group received purified buffer solution (PBS-) using the same osmotic pump. The bone formation of lengthening segment evaluated through radiograph measurement, weekly. The bone mineral content (BMC) and bone mineral density (BMD) of lengthened segment, surrounding segment of operated and contra-lateral tibiae measured by DXA weekly. Rabbits were sacrificed at 4, 6, and 8 weeks after operation for examination by pQCT and three- point bend test.
Radiographic evaluation indicated osteopenia significantly prevented in alendronate treated group compared with the PBS- treated group. The BMC of the lengthened segment was 7 times higher in alendronate treated group at 8 weeks (P<0.0001). The mean areal BMD (g/cm²) at 8 weeks in the lengthened 83% (p<0.0001), in the proximal to the lengthened segment 37% (p<0.001), and the distal to the lengthened segment 43% (p<0.001) were higher in alendronate treated group. The mean volumetric density (BMD) in the lengthened segment at 4 weeks 12% (p=0.35), 6 weeks 89% (p<0.003), and 8 weeks 94% (p<0.001) higher in alendronate treated group. In the proximal and the distal to the lengthened segment at 6 and 8 weeks were significantly higher in alendronate treated group (p<0.001). Three-point bending test demonstrated that alendronate treated bone at 6 and 8 weeks were significantly stronger than PBS- treated group.
This study has demonstrated that continuous local injection of alendronate into the lengthened segment can prevent osteopenia during distraction osteogenesis.

Disclosures: ***A. Abbaspour****, None.*

SU002

Dried Plum Polyphenols Increase Insulin-like Growth Factor I (IGF-I) Production in Osteoblast-like Cells. S. Hooshmand, S. C. Chai*, B. H. Arjmandi. Nutrition, Food and Exercise Sciences, Florida State University, Tallahassee, FL, USA.

Osteoblast-like cells (MC3T3-E1) secrete several growth regulating factors include insulin like growth factor I (IGF-I), transforming growth factor beta (TGF-ß) and IGF-II in descending order of abundance. It has been shown that these growth factors produced by MC3T3-E1 have autocrine actions on these cells. Amarnani et al. showed that exogenous addition of IGF-I and IGF-II stimulates MC3T3-E1 cell proliferation; however, TGF-ß inhibited osteoblast proliferation. IGF-I not only causes osteoblast proliferation but also enhances bone
matrix proteins synthesis in these cells. Our earlier clinical study showed that consumption of dried plum increase IGF-I level in serum of osteopenic postmenopausal women. To investigate the mechanism of action of this observation, we treated MC3T3-E1 cells with 0, 10, 100 and 1000 μg/ml dried plum extract for 3, 6, 9, 12 and 15 days. Media were collected every three day to measure alkaline phosphatase (ALP) activity and IGF-I. At the end of 15 day period, RNA was extracted to further measure the mRNA expressions of ALP and collagen. Dried plum extract was able to increase IGF-I significantly in dose of 1000 μg/ml in days of 12 and 15 compare to control group. ALP increased numerically in dose of 1000 μg/ml in day 15. However, this increase in ALP was not significant. Changes in mRNA levels of ALP and collagen will be reported at the time of poster presentation. The findings of this study suggest that polyphenols in dried plum play important role in bone formation.

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