6th International Conference on Osteoporosis and Bone Research: ‘Poster Presentation’ Abstracts
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Poster Presentation Abstracts

PP 01
Quantification of Visceral Adipose Tissue Using Lunar DXA
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Background and Purpose: Dual-energy X-ray absorptiometry (DXA) is considered to be the gold standard for the assessment of osteoporosis and other skeletal disorders. DXA technology also allows for accurate measurement of body composition with high precision, low X-ray exposure, and short scanning time. Recently, a new application to quantify visceral adipose tissue (VAT) over the android region of a total-body DXA scan has been described, and was reported to have a high correlation with computed tomography (CT) in a Caucasian population. This application is of particular interest in Asian populations, as they are more susceptible to the accumulation of VAT and usually have higher percentages of VAT with respect to total body fat when compared to Caucasians. The purpose of this study is to evaluate the performance of DXA VAT in an Asian population.

Methods: A total of 145 adult Asian male and female volunteers with an age range of 19–83 and BMI range of 18.5–39.3 kg m⁻² were studied using both DXA and CT. The agreement between DXA and CT was evaluated using a paired t-test, correlation test and Bland–Altman analysis. The results were compared to those of a previous study performed on a Caucasian population.

Results: CT and DXA VAT measurements were highly correlated, with a coefficient of determination (r²) for regression values of 0.947 for females, 0.981 for males and 0.915 (95% CI: 0.940–0.969) combined. Bland–Altman analysis showed a moderate bias of 143 cm⁻³ for females and 379 cm⁻³ for males. Combined, the bias was 262 cm⁻³, with 95% limits of agreement of −232 to 755 cm⁻³. Further linear discriminant analysis was performed on CT data from both the Caucasian and Asian data sets to better understand the bias. Analysis showed that by using the fixed abdomen diaphragm position, as determined by CT breath-hold status, 90% of subjects could be correctly assigned to their respective CT acquisition methods. This demonstrated that a significant portion of the observed bias could be largely due to VAT movement as a result of the CT breath-hold status.

Conclusion: We conclude that VAT can be measured in the Asian population using DXA. In future studies comparing DXA VAT measurement to reference standards such as CT, the breath-holding protocol for the reference method must be carefully controlled.

PP 02
Distinct Tissue Mineral Density (TMD) Distribution in Human Trabecular Plates and Rods
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Objective: A newly developed individual trabecular mineralization (ITM) technique provides unique measures of individual trabecular plate or rod mineralization—one of the critical determinants of bone strength. We hypothesized the distinct distributions of individual trabecular TMD (Tb.TMD) with trabecular types and orientations. The objectives of this study were (1) to investigate the three-dimensional TMD distribution in human trabecular bone by performing ITM analyses on BOTH conventional µCT scans and SRµCT scans—the gold standard for evaluating TMD; and (2) to quantitatively evaluate the deviation of µCT-based TMD assessment compared with SRµCT due to the inherent limitation of polychromatic µCT (for example, beam-hardening effect).

Methods: 14 trabecular bone cylinders were harvested from femoral heads, vertebrae and proximal tibiae, and imaged by µCT at 8µm (Scanco µCT40). Images were reconstructed and beam hardening corrected (BHC) based on a hydroxyapatite (HA) wedge phantom of 200 or 1200 mg HAlcm⁻³. SRµCT imaging was performed at 7.5µm at the National Synchrotron Light Source. Attenuation values were converted to HA density by phantom calibration. The microstructure of a cubical subvolume of each bone core was segmented into individual trabeculae, and Tb.TMD was calculated for each trabecula. Mean Tb.TMDs of trabecular plates and rods were examined, as well as stratified to various orientations.

Results: SRµCT-based ITM measurements showed that Tb plates were 1.0% more mineralized than rods, which was consistent with another previous µCT-based study with 63 samples. However, the SRµCT and µCT of the current 14 trabecular samples showed significantly different Tb.TMD measurements. µCT with BHCs resulted in lowering of the plate and rod Tb.TMD SRµCT values by 12.2–17.0% and 11.4–16.2%, respectively. Owing to underestimation of plate Tb.TMD, the plate vs rod Tb.TMD difference was diminished in µCT-based
mechanical properties. Results also suggested that plate Tb.TMD varied with trabecular orientations in a consistent pattern: transverse-oblique-longitudinal, which was not influenced by µCT artifacts. Interestingly, axially aligned plates were less mineralized than plates along other directions, though they account for the majority of the bone volume and predominate in mechanical properties. 

**Conclusion:** The trabecular type and orientation-associated heterogeneous TMD distribution in trabecular bone is confirmed by SΡµCT-based ITM analysis. Trabecular plates, particularly transverse plates, are most highly mineralized. µCT-based ITM analysis is feasible with adequately large sample size and appropriate BHC.

**PP 03**

**Varied Perfusion at Different Vertebral Levels of Lumbar Spine**

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**Objective:** A previous study reported decreased bone perfusion in the lower lumbar vertebra compared to upper lumbar segments. However, no systematic study has investigated the relationship between the variation in BMD and bone perfusion among the lumbar vertebrae. The objective of this study is to characterize bone perfusion in lumbar spine as a function of level and anatomic location. Through semiquantitative and quantitative parameters, the BMD and site-specific bone microcirculation characteristics are compared among different levels of lumbar spine.

**Methods:** Fourteen subjects (age 72.1±4 years) in total were involved in this study. The BMD of L1–L4 levels was measured by dual-energy X-ray absorptiometry (DXA). Dynamic contrast enhancement MRI (DCE-MRI) data were acquired in the mid-lumbar sagittal plane. The region of interest (ROI) encompassing the cancellous bone of vertebral body of L1–L4 was drawn manually on the DCE MRI image to obtain the characteristic signal. A pharmacokinetic model was employed to analyze the DCE MRI characteristic curve. Two parameters were analyzed: A*k<sub>ep</sub>, the permeability between the interstitial space and plasma, and S<sub>max</sub>, the maximum enhancement.

**Results:** BMD, A*k<sub>ep</sub> and S<sub>max</sub> showed statistically non-significant difference among vertebral levels. A gradually increasing trend for BMD and decreasing trend for A*k<sub>ep</sub> and S<sub>max</sub> were observed from upper to lower lumbar levels, respectively. In other words, the lower lumbar vertebrae have higher BMD while with lower bone perfusion. Meanwhile, the three investigated parameters showed a good linear relationship with the vertebral levels.

**Conclusions:** The current study has basically reconfirmed the previous study’s findings. Further, we found that both perfusion and BMD displayed a linear correlation to the vertebral level. However, the changing trend for BMD and perfusion was inverse. This finding indicates that denser mineral content in the bone could result in a decreased perfusion. However, the correlation of the vertebral level with BMD is not as significant as that with perfusion parameters. Other factors, such as the percentage of yellow marrow content, are possibly varied along the lumbar spine levels.

**PP 4**

**MiR-125b Suppresses the Proliferation and Osteogenic Differentiation of Human Bone Marrow-Derived Mesenchymal Stem Cells**

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The decreased biological function of human bone marrow-derived mesenchymal stem cells (hBMSCs) with aging is one of the key factors leading to the decreasing bone mass and senile osteoporosis. MicroRNAs (miRs) are noncoding small RNAs involved in gene regulation. It is still unclear whether some microRNA(s) are involved in the progression of osteoporosis by regulating BMSC function.

We found that miR-125b expression levels were much higher in hBMSCs isolated from elderly patients with osteoporotic fractures than in those from normal younger persons (P<0.05). Function studies showed that overexpression of miR-125b suppressed the proliferation and osteoblast differentiation of senile hBMSCs. In contrast, inhibition of miR-125b promoted the proliferation and osteoblast differentiation of senile hBMSCs. Further data suggested that miR-125b might regulate osteoblast differentiation by modulating the expression of Oxs. Taken together, our results indicated the essential role of miR-125b in regulating the proliferation and osteoblast differentiation of hBMSCs. We concluded that miR-125b might be a potential target to treat senile osteoporosis.

**PP 5**

**Rodent Trabecular Bone Marrow is Enriched with a Highly Proliferative, Immunosuppressive and PTH-Responsive Population of Mesenchymal Progenitors**

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The decreased biological function of rodent bone marrow (BM) by simply flushing long bones yields mesenchymal stem cells (MSCs) from the diaphyseal (central) bone but not those residing in the trabecular (endosteal) bone.

**Methods:** We have developed a unique method based on enzymatic digestion of rat or mouse bone tissues to harvest BM cells and generate MSCs from the endosteal and central marrow regions. Endosteal MSC numbers, cell surface marker expression, differentiation potential, proliferation rate, cell cycle inhibitor gene expression and immunosuppressive activity were characterized by CFU-F assays, flow cytometry, in vitro differentiation assays, MTT assays, cell counting, real-time PCR, in vitro T cells apoptosis assays, and an acute colitis mouse model.

**Objective:** The traditional method of harvesting rodent bone marrow (BM) by simply flushing long bones yields mesenchymal stem cells (MSCs) from the diaphyseal (central) bone but not those residing in the trabecular (endosteal) bone.

**Methods:** We have developed a unique method based on enzymatic digestion of rat or mouse bone tissues to harvest BM cells and generate MSCs from the endosteal and central marrow regions. Endosteal MSC numbers, cell surface marker expression, differentiation potential, proliferation rate, cell cycle inhibitor gene expression and immunosuppressive activity were characterized by CFU-F assays, flow cytometry, in vitro differentiation assays, MTT assays, cell counting, real-time PCR, in vitro T cell apoptosis assays, and an acute colitis mouse model.
Results: Endosteal BM from 1-month-old rats formed 3-fold more and 1.7-fold larger CFU-F colonies with a similar percentage of alkaline phosphatase (ALP)-positive colonies compared to the central BM. Similar results were observed with mice, and isolation of endosteal BM cells from mice expressing GFP under a 2.3-kb collagen 1 promoter confirmed that the GFP+ osteoblasts, released by digestion into the endosteal BM, were not the source of the endosteal CFU-F colonies. Endosteal MSCs generated by this method expressed traditional BM MSC surface markers and were capable of multi-lineage differentiation. Interestingly, they exhibited accelerated growth rate with a doubling time of about 20 h compared to central MSCs (~48 h), which is likely due to the decreased expression of the cell cycle inhibitors p15, p16 and p21. Moreover, they showed greater immunosuppressive activity in an in vitro T cell apoptosis assay and a dextran sulfate sodium-induced acute colitis mouse model. Further experiments revealed a 2-fold decrease in endosteal CFU-F colony number in aging mice compared to young mice, which is consistent with a 5-fold decrease in the percentage of Sca1high+CD29high+CD45− mesenchymal progenitors in the endosteal BM in aging mice. Parathyroid hormone (PTH) injection is the only approved anabolic therapy for osteoporosis and it greatly stimulates bone formation. A single PTH injection significantly increased the number (3-fold) of CFU-F colonies and the size (2.1-fold) of CFU-ALP-negative colonies formed from the endosteal BM, with no effect on those from the central BM.

Conclusion: We have provided strong evidence that endosteal MSCs from bone have distinct functional characteristics from the central MSCs traditionally used in research and that they are more metabolically active and relevant to physiological bone formation.

PP 7
Glucocorticoids alter the balance between osteogenesis and adipogenesis of MSCs by regulating DNA methylation of C/EBPalpha
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Aims: The balance between osteogenesis and adipogenesis is disrupted in many diseases, including glucocorticoid (GC)-induced osteoporosis. Both in vivo and in vitro experiments have demonstrated that dexamethasone (Dex), a synthetic GC, may alter the balance, but the underlying mechanism has not been elucidated. Our previous research proved that downregulation of CCAAT/enhancer binding protein (C/EBP) alpha, the key regulator of adipogenesis, by DNA hypermethylation at the late stage of osteogenesis is one of the regulatory mechanisms for the differentiation balance between osteoblast and adipocyte. In this study, we investigated the possibility that Dex might shift mesenchymal stem cell (MSC) differentiation to favor the adipocytes over osteoblasts by altering C/EBPalpha methylation status.

Methods: We established an osteoblast differentiation model and a trans-differentiation model of osteoblasts to adipocytes in the mouse mesenchymal cell line C3H10T1/2 and tested whether C/EBPalpha methylation status is changed or not. In order to test whether the canonical Wnt pathway works downstream of Dex to affect the balance between osteogenesis and adipogenesis, as well as C/EBPalpha methylation, we investigated if this pathway is inhibited by Dex in our cell model and investigated the molecular mechanisms by which Dex and Wnt regulate C/EBPalpha methylation.

Results: We found that Dex indeed prevented osteoblastic differentiation of C3H10T1/2 while improving adipogenesis. C/EBPalpha maintained low methylation and was highly expressed at the late stage of osteogenesis. Moreover, we found that stimulating the Wnt/beta-catenin pathway (LiCl) could partly rescue the effect of Dex on osteogenesis and C/EBPalpha methylation. We also found that the molecular mechanism of regulating C/EBPalpha methylation by Dex or LiCl is by affecting Dnmt3a/b binding to its promoter.

Conclusion: C/EBPalpha methylation is an important process in BMP2-induced osteogenesis. One of the mechanisms by which Dex disrupted the balance between osteogenesis and adipogenesis was by promoting C/EBPalpha expression by inhibiting DNA methylation induced by BMP2. Even though the Wnt/beta-catenin signaling pathway alone cannot induce DNA methylation of C/EBPalpha, as a downstream pathway for BMP2, canonical Wnt signaling is an indispensable part of C/EBPalpha methylation. Investigation of the mechanisms of C/EBPalpha methylation might reveal some drug targets to treat GC-induced bone loss.

PP 8
Perfusion Distribution Change in Vertebra with Modic Changes
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Objective: Modic changes are a common phenomenon on magnetic resonance imaging in spinal degenerative diseases and are strongly linked with low back pain. There are three types of Modic changes: type I, II and III, classified by different signal change patterns in the MRI images as summarized by Modic et al. Most of the current studies on Modic change focus on its histopathologic mechanism, type development and transformation, etc. However, no study has shown if blood supply changes in the bone marrow with Modic changes. Dynamic contrast enhanced (DCE) MRI is a good approach to investigate the blood perfusion in living tissues. The purpose of this study is to investigate the perfusion in the bone marrow with Modic changes through DCE-MRI.

Methods: Twenty-four elderly male subjects (age 72.8±3.3 years) were included in this study, including 15 patients with Modic changes (type I, II, III) and 9 normal subjects with no degenerative disease throughout the lumbar spine. DCE scan was conducted in the mid-lumbar sagittal plane. A region of interest (ROI) was drawn manually for each Modic change and normal bone marrow area on the vertebra, where the signal intensity curves were extracted pixel-by-pixel in the ROI. Brix model was employed to analyze the perfusion curves. The normalized fitted curves were divided into three patterns distinguished by the slope of the end of the curve.
Results: Modic type I showed higher ROI pattern percentage (P<0.05) and normalized pattern 1 percentage (P=0.000); Modic type II showed a lower ROI pattern percentage (P=0.000); while Modic type III showed no significant difference. The Normal group had a higher normalized pattern 3 percentage compared with Modic groups (P<0.01).

Conclusions: The blood perfusion varied in different Modic changes. Most literatures have reported that Modic change type II is a fatty marrow. The lower ROI pattern percentage indicates weak perfusion ability in the type II region. The higher percentage of pattern 3 indicates that the perfusion ability of normal subjects is stronger than that of patients with Modic change. This study indicated that the perfusion ability of the bone marrow could distinguish Modic change regions with normal regions and the result corresponded with the pathogenetic mechanism of Modic change.

PP 9
Bone Marrow Perfusion Distribution of Proximal Femur in Subjects with Different BMD
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Objective: A previous DCE-MRI study on proximal femur showed reduced blood perfusion in osteopenic and osteoporotic bone compared to that with normal bone mineral density (BMD). This study aimed at investigating the blood perfusion distribution of bone marrow at proximal femur in subjects with varying BMD to increase our knowledge of the blood perfusion and vascularization anomalies occurring at osteoporotic proximal femur.

Methods: The final cohort included 87 female subjects (age=71±4.2 years). Dynamic contrast enhancement (DCE) MRI data were acquired in an oblique coronal plane aligned along the midportion of the proximal femur. DCE-MRI curve for each pixel was extracted and fitted by a pharmacokinetic model and then classified into three patterns: Pattern 1, fast enhancement followed by a slow enhancement; Pattern 2, fast enhancement followed by a signal plateau; Pattern 3, fast enhancement followed by a quick washout. The proximal femurs were divided into four regions of interest (ROIs) manually (head, neck, intertrochanteric area and shaft). The BMD of proximal femur was measured by dual X-ray absorptiometry (DXA), based on which the subjects were divided into three groups (normal, osteopenic, and osteoporotic).

Results: The pattern coloring rate was employed to quantify the perfusion distribution. For all subjects, the overall coloring rate showed a significant difference (P<0.001) among ROIs, where the femoral head and shaft had the lowest and highest coloring rate, respectively. For the groups with different BMDs, normal subjects had a significantly higher overall coloring rate than the other two groups for all investigated ROIs (P<0.05). For the perfusion pattern distribution, the intertrochanteric area showed a higher perfusion pattern gradient than the other three ROIs.

Conclusions: First, a notable reduction in overall pattern coloring rate in subjects with reduced BMD was observed, indicating that the blood perfusion decreased as a whole in the development of osteoporosis at proximal femur. Second, femoral head and shaft had the lowest and highest blood perfusion, respectively. Thirdly and interestingly, it is obvious for all three BMD groups that except for the intertrochanteric line, the perfusion decreases significantly from the lesser trochanter to the greater trochanter. As intertrochanteric fracture is one of the most common fractures at the hip, such a blood perfusion distribution manner may be one of its underlying mechanisms.

PP 10
Aquaporin 1 regulates MSC cell migration in vitro and in vivo
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Objective: Administration of bone marrow mesenchymal stem cells (MSCs) is widely used in bone defect repair and fracture healing. Aquaporin 1 (Aqp1) belongs to the aquaporins family of water-specific, membrane-channel proteins, which is also proposed to promote tumor angiogenesis. To enhance the recruitment efficiency of MSCs to the injury sites, we manipulated the expression of Aqp1 gene in MSCs and explored its effects on MSC migration both in vitro and in vivo.

Methods: MSCs were isolated from rat bone marrow, characterized and expanded in vitro. Aqp1 overexpression and knocking down of stable cell lines were established by lentiviral transfection, which were used for cell migration assessment through transwell and wound-healing assays. GFP-labeled Aqp1-overexpressing MSCs and GFP-MSCs were then administered systemically via tail vein injection in rats with experimental tibia fracture. The percentage of GFP-expressing cells at fracture sites was quantified and compared statistically. Other experiments to address the underlying mechanisms were also conducted by western blot, co-immunoprecipitation and confocal image techniques.

Results: Knocking down Aqp1 had no effects on osteogenesis, adipogenesis, chondrogenesis and proliferation of MSCs. Overexpression of Aqp1 promoted MSC migration, while knocking down Aqp1 impaired MSC migration in vitro. Higher numbers of GFP-MSCs were found at the fracture site in the Aqp1-MSCs-treated group compared to the GFP-MSCs group. Expression of beta-catenin and focal adhesion kinase (FAK) was upregulated in the Aqp1-MSCs, and downregulated in the Aqp1-knocking-down MSCs. Beta-catenin and FAK were co-immunoprecipitated with Aqp1, and the co-localization of FAK and Aqp1 was confirmed by confocal images.

Discussion: This study demonstrates that Aqp1 enhances MSC migration ability by affecting expression of beta-catenin and FAK. Our findings suggest a novel function of Aqp1 in governing MSC migration, which may have therapeutic potential.
Objective: Reduced bone marrow perfusion has been found in osteoporotic bone by using dynamic contrast enhancement (DCE)-MRI. However, previous quantitative studies on bone perfusion did not include the arterial input function (AIF), which is one determinant of perfusion function. There were studies reporting the importance of AIF selection in the pharmacokinetic analysis of DCE-MRI. The objective of this study is to characterize bone marrow perfusion properties through DCE-MRI in a quantitative way by including personalized AIF.

Methods: Eighty-two male subjects (age 72.4±3.6 years) were involved in this retrospective study. DCE MRI data were acquired at the mid-L3 vertebrae of the lumbar spine. The region of interest (ROI) was drawn manually by encompassing the cancellous bone of the vertebral body to obtain the time-signal intensity curve. A modified Tofts model, which adopted a personalized AIF model, was employed to analyze the time-signal intensity curve. The subjects were classified into three groups (normal, osteopenia and osteoporosis) according to the T-score of L3 vertebrae. Quantitative parameters, Ktrans (extravasation transfer constant), ve (extravascular extracellular space, EES) and vp (plasma volume), were extracted and compared among the three groups.

Results: Analysis of variance (ANOVA) showed no significant difference in age (P=0.297) among the three groups. For the pharmacokinetic parameters, a significant decrease was observed for Ktrans (P=0.005) and ve (P=0.001) in the osteopenia and osteoporosis groups, while the variation was non-significant for vp (P=0.667) among the three groups with different BMD.

Conclusions: The parameter Ktrans is the extravasation transfer constant. Its reduction implies a degenerated blood vessel function, which could indicate a decreased vessel wall permeability. Such reduction could also diminish the nutrition exchange between the bone tissue and the vessel. Parameter ve, an indication of the interstitial space, was also decreased in osteoporotic bone. When the bone mineral content is lost, there must be changes in bone marrow content that result in a smaller interstitial space. In our previous study, such change was most probably due to the marrow fat content increase. The diminished interstitial space would inversely affect the blood supply to bone tissue. However, the plasma volume vp showed a non-significant change in bone with lower BMD, implying that the capillary density or the arterial blood supply system may not change with the development of osteoporosis in terms of the volume.

Objective: Recently studies have suggested that SAON may be a disease of bone cells and/or mesenchymal stem cells (MSCs). We previously reported that Icaritin, an intestinal metabolite of epimedium-derived flavonoids (EFs) could reduce SAON incidence with inhibition of both thrombosis and lipid deposition, but the detailed mechanism remains unclear. In this study, we investigated the effect of Icaritin on the differentiation potential of MSCs derived from both normal and SAON rabbits.

Methods: An SAON model in rabbit was established by following a standard protocol. Bone marrow MSCs were aspirated from the proximal femur of normal and SAON rabbits. MTT assay was performed to test cell proliferation. ALP activity assay, ALP staining, Alizarin Red S staining and Oil red O staining were used to evaluate the differentiation potential of MSCs. Real-time PCR and western blotting were performed to detect RNA and protein expression.

Results: Differentiation assay showed that the osteogenic differentiation potential declined while adipogenic differentiation ability was elevated in MSCs derived from SAON rabbits. Icaritin enhanced the osteogenic differentiation of MSCs from normal and SAON rabbits in a dose-dependent manner. Icaritin upregulated Cola1, BMP2, Runx2 and osteocalcin mRNA expression during osteogenic differentiation of MSCs derived from both normal and SAON rabbits. Icaritin inhibited adipogenic differentiation of MSCs derived from both normal and SAON rabbits in a dose-dependent manner. Icaritin inhibited adipogenic differentiation of MSCs derived from both normal and SAON rabbits in a dose-dependent manner. Icaritin enhanced the osteogenic differentiation of MSCs from normal and SAON rabbits in a dose-dependent manner. Icaritin enhanced the osteogenic differentiation of MSCs from normal and SAON rabbits in a dose-dependent manner. Icaritin enhanced the osteogenic differentiation of MSCs from normal and SAON rabbits in a dose-dependent manner. Icaritin enhanced the osteogenic differentiation of MSCs from normal and SAON rabbits in a dose-dependent manner.

Conclusion: An imbalance between osteogenic and adipogenic differentiation was found in MSCs derived from SAON rabbits; Icaritin could enhance the osteogenic differentiation of the MSCs in normal rabbits and partly rescue osteogenic differentiation in SAON rabbits.
Results: Micro-CT study: Promoted bone formation and decreased porosis at the osteochondral junction
A significantly larger joint space was detected in DH strain guinea pigs at 1 and 2 months than BS2. Both strains showed decreased joint space with time. Elevated bone formation and higher bone mass were detected at the osteochondral junction in the DH group, compared with BS2. The BMD of the osteochondral junction in the DH group is higher than that in the BS2 group at 1, 2 and 3 months, although the difference at 1 and 2 months was not significant. The thickness of the osteochondral junction is found to be significantly higher in the DH group than in the BS2 group at 2 and 3 months. Additionally, the porosity of the osteochondral junction is significantly lower in the DH group at 1, 2 and 3 months. The porosity at the osteochondral junction in both strains showed decreased change with time.

Histological findings: Cartilage hypercellularity, hypertrophic chondrocyte clustering and thickened calcified cartilage layer
Significantly higher chondrocyte density was identified in DH guinea pigs than in BS2 guinea pigs at 1, 2 and 3 months. The morphology of chondrocytes in the DH strain appeared to be hypertrophic at 1 and 2 months compared to BS2. Hypertrophic chondrocyte clustering was observed at 3 months in the DH strain. The thickness ratio of the calcified cartilage (CC) layer to the non-calcified cartilage layer (NCC) decreased significantly in the DH strain, while in comparison no significant change of thickness ratio (CC/NCC) was observed in the BS2 group.

When we quantified the thickness of the calcified cartilage layer (CC), the subchondral bone plate (SBP) and the osteochondral junction (CC+SBP), we found that the thickness of the CC layer in the DH group is significantly higher than that of BS2 at 1, 2 and 3 months. While the thickness of SBP in the DH group was lesser than that of the BS2 group, the difference was not significant. Also, the total thickness of the osteochondral junction in DH was significantly higher than BS2, which was in accordance with the results recorded from micro-CT.

Conclusion: The decreased porosity of the osteochondral junction may impair the molecular interaction between cartilage and subchondral bone, thus leading to early cartilage degeneration of the Dunkin–Hartley guinea pig spontaneous osteoarthritis model.

PP 14
Clinical Features and Mutations of 1α-Hydroxylase Gene (CYP27B1) in Seven Chinese Families with PDDR
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Objective: Pseudovitamin D-deficiency rickets (PDDR) is an autosomal recessive disorder resulting from a defect in renal 25-hydroxyvitamin D 1α-hydroxylase (CYP27B1), the key enzyme in vitamin D metabolism. This study tries to analyze the clinical features of PDDR and to detect mutations of the CYP27B1 gene in seven Chinese families with PDDR.

Methods: Nine PDDR patients from seven unrelated Chinese families were recruited. The clinical diagnosis was confirmed by medical history, physical examinations, laboratory results and radiological features. CYP27B1 mutations were detected by direct DNA sequence analysis.

Results: PDDR patients were characterized by the early onset and a severe syndrome of rickets. Nine different mutations of CYP27B1 gene were identified. Two of them are novel missense mutations; two are novel nonsense mutations; two are novel deletion mutations; two are recurrent deletion mutations. c48-60del and c1446delA and an insertion c1325–1332insCCCACCC were published previously. Three cases from two families are homozygous c1325–1332insCCCACCC mutations. The other cases had a compound heterozygous status, and their parents were heterozygous carriers of these mutations.

Conclusions: The study describes six novel mutations in the CYP27B1 gene and shows the correlation between these mutations and the clinical findings in 1α-hydroxylase deficiencies.

PP 15
Identification of One Recurrent Mutation in the TRAPPC2 Gene in a Large Chinese Family with Spondyloepiphyseal Dysplasia Tarda
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Introduction: X-linked spondyloepiphyseal dysplasia tarda (SEDT; MIM 313400) is a rare X-linked recessive osteochondrodysplasia. The major features of the disorder include disproportionate (short-trunked) short stature first evident in childhood between 5 and 14 years, shortness due to impaired growth of the spine, radiologically, characteristic flattening of vertebrae with central and posterior humping, dysplastic changes of femoral heads and neck, and minor changes in other bones. It arises from mutations in the tracking protein particle complex subunit 2 gene (TRAPPC2) that encodes a 140-amino-acid protein, sedlin, with a putative role in endoplasmic reticulum (ER)-to-Golgi vesicular transport.
Method: A four-generation Chinese family with three members affected by SEDT was investigated. Mutation screening of the TRAPPC2 gene was carried out.

Results: We screened for TRAPPC2 gene mutations and identified that the proband (III6) and his nephew (IV1) with SEDT carried a recurrent deletion mutation (c.271_275delCAAGA) of TRAPPC2 that leads to frameshift and a premature termination (p.Q91del; E92fsX100) (Figure 3). The proband’s daughter (IV6) and niece (IV5) were proved to be the carriers of this mutation. The proband’s wife did not carry this mutation. This mutation was not detected in 200 healthy unrelated controls.

Conclusion: This is the first report of this recurrent mutation of TRAPPC2 in Chinese population with SEDT and is helpful in early molecular diagnoses of SEDT.

PP 16
Characterization of Subchondral Plate Porosity of Tibia Plateau from Patients with Late Osteoarthritis Using Micro Computed Tomography
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Purpose: The subchondral plate facilitates normal cross-talk between articular cartilage and trabecular subchondral bone. In osteoarthritis (OA), changes, especially the porosity, occurring in subchondral plate may disturb cross-talk homeostasis, which is possibly a principal cause for OA occurring and developing. To investigate these changes, we examined the subchondral plate of human samples using sensitive microcomputed tomography (micro-CT).

Methods: Tibia plateaus were collected from patients undergoing total knee replacement because of OA in Hong Kong from 2011 to 2012, and those having a relatively intact lateral side and the remaining mid-1/3 medial side were selected. Thus, the total number of Tibia plateaus was 32, of which 22 were from female and 10 from male, with a mean age of 71 (71±9) years. An area of 10cm×10cm of the middle of both lateral and medial sides, which is the most weight-bearing region, is the targeted area. The first region of interest (ROI) was defined as the subchondral plate, and the second ROI was defined as a depth of 2mm from the surface downwards.

Results: The average (Ave) and s.d. of the two ROIs

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<tr>
<th>Structural bone indices</th>
<th>Lateral (average±s.d.)</th>
<th>Medial (average±s.d.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n Percent of porosity (%)</td>
<td>Subchondral plate</td>
<td>32.34±10.82</td>
</tr>
<tr>
<td></td>
<td>2 mm</td>
<td>61.31±7.34</td>
</tr>
<tr>
<td>Volume of pore space (mm³)</td>
<td>Subchondral plate</td>
<td>20.1±8.93</td>
</tr>
<tr>
<td></td>
<td>2 mm</td>
<td>105.94±13.59</td>
</tr>
<tr>
<td>Percent of bone volume (BV/TV) (%)</td>
<td>Subchondral plate</td>
<td>63.66±14.57</td>
</tr>
<tr>
<td></td>
<td>2 mm</td>
<td>38.69±7.34</td>
</tr>
</tbody>
</table>

P<0.01, compared with the lateral side, paired-samples t-test.

Conclusion: The current study firstly provides quantitative data on the structural changes in the subchondral plate of the tibia plateau from patients with OA. The changes in plate porosity may increase mutual interaction between subchondral trabeculae, bone marrow cells and the articular cartilage, leading to development of OA.

PP 17
Identification of One Novel Mutation in the EVC2 Gene in a Chinese Family with Ellis–van Creveld Syndrome
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Introduction: Ellis–van Creveld syndrome (EVC, MIM 225500) is an autosomal recessive skeletal dysplasia characterized by short limbs, short ribs, postaxial polydactyly, and dysplastic nails and teeth. Congenital cardiac defects, most commonly a defect of primary atrial septation producing a common atrium, occur in 60% of affected individuals. The prevalence of EVC is estimated to be 7 per 1 million births in non-Amish population.

Method: One affected individual with EVC and his parents were clinically studied. The EVC and EVC2 genes were screened and analyzed, and the mutations were confirmed using molecular genetic techniques.

Results: The affected individual had novel compound heterozygous EVC2 R399X/W828X mutations, inherited from the patient’s unaffected mother and father, respectively. No mutations were identified in the EVC gene. The R399X and W828X mutations were not found in DNA samples from 250 healthy volunteers.

Conclusions: We identified a novel EVC2 mutation (W828X) in an affected Chinese individual with EVC. Our findings may assist not only in the clinical diagnosis of EVC but also in the interpretation of genetic information used for prenatal diagnosis and genetic counseling.

PP 18
Mutational Survey of the PHEX Gene in 10 Unrelated Chinese Families with X-linked Hypophosphatemic Rickets/Osteomalacia
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Objective: X-linked dominant hypophosphatemia (XLH) is the most prevalent form of the inherited rickets in human. The aim of our study is to identify mutations of the PHEX gene in 10 unrelated Chinese families and two sporadic patients with hypophosphatemic rickets/osteomalacia.

Methods: 10 unrelated Chinese Han nationality families, including 45 individuals, two sporadic patients and 250 healthy donors, were recruited and their genomic DNA samples were extracted. Hypophosphatemic rickets/osteomalacia were diagnosed based on the clinical manifestations, physical examinations, characteristics of their bones on X-ray and laboratory results. All 22 exons and their exon–intron boundaries of the PHEX gene were amplified by polymerase chain reaction (PCR) and sequenced directly.
Results: We identified six novel mutations in the 10 unrelated families. (1) In family 1: the proband (II1, 5-year-old girl) and her father (I1, 34-year-old male) carried a novel nonsense mutation c.1119G>A in exon 6 resulting in p.W373X. (2) In family 2: the proband (II1, 4-year-old girl) and her mother (II2, 27-year-old female) carried a novel missense mutation c.1751A>C in exon 17 resulting in p.H584P. (3) In family 3: the proband (II2, 43-year-old female) and her mother (II3, 73-year-old female) carried a novel nonsense mutation c.1332G>A in exon 12 resulting in p.W444X. (4) In family 4: the proband (III2, 21-year-old female) and her sister (III1, 23-year-old female) carried a missense mutation c.1601C>T in exon 15 resulting in p.P534L. (5) In family 5: the proband (IV6, 10-year-old boy) and his mother (III6, 44-year-old female) carried a novel frameshift mutation c.2033dupT in exon 20 resulting in p.T679H. (6) In family 6: the proband (II3, 53-year-old male) carried a novel nonsense mutation c.1294A>T in exon 11 resulting in p.K432X. (7) In family 7: the proband (III4, 23-year-old female) carried a novel missense mutation c.2192T>C in exon 22 resulting in p.F731S. (8) In family 8: the proband (II1, 14-year-old boy) and his mother (II2, 33-year-old female) carried a splicing mutation c.1174-1G>A in intron 10. (10) In family 10: the proband (II1, 40-year-old female) and her mother (II2, 59-year-old female) carried a deletion mutation c.1694delA in exon 16 resulting in p.Y565F. (11) The two sporadic cases: the proband (II1, 16-year-old male) carried a splicing mutation c.1768+2T>G in intron 17 and the proband (II1, 3-year-old boy) carried a deletion mutation c.2154_2169delinsA in exon 22 resulting in p.N718_N723delinsK. (12) No mutation was found in 250 healthy controls.

Conclusions: Our study enriched the PHEX gene mutation types in Chinese people with X-linked dominant hypophosphatemic rickets/osteomalacia, which are useful to understand the genetic basis of Chinese patients with XLH.

PP 19
Novel TRPM6 Mutations in a Chinese family with Primary Hypomagnesemia and Secondary Hypocalcemia
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Hypomagnesemia with secondary hypocalcemia (HSH) is a rare inherited autosomal recessive disease, characterized by very low serum magnesium and calcium concentrations and neurological symptoms. Recently, mutations in the TRPM6 gene coding for TRPM6 were found to be responsible for this disease. To elucidate the characteristics of TRPM6 gene mutations in Chinese patients with HSH, we analyzed the TRPM6 gene in one Chinese family with two affected family members.

Subjects and Methods: We observed a Chinese family of the Han ethnic group consisting of four family members. The proband and her sister presented with the same manifestation, such as generalized seizures and muscle spasms, and their serum magnesium and calcium levels were very low. The oldest affected children of the family died at 1 year and 8 months old. The parents are nonconsanguineous and unaffected. The study has been approved by the Department of Scientific Research of Peking Union Medical College Hospital. All participants were given informed consent before study participation.

Blood samples of family members were collected after an overnight fast. The biochemical parameters were measured in patients and their parents. 24-h urine magnesium, urine calcium and urine creatinine were measured by spectrophotometry using routine assays. Genomic DNA was extracted from peripheral white blood cells. All 39 exons of the TRPM6 gene and their corresponding intron-exon boundaries were amplified by polymerase chain reaction. The amplification products were purified and sequenced by an automated sequencer according to the manufacturer's protocol.

Results: Both patients were compound heterozygotes for the TRPM6 gene mutations. The mutational spectrum comprised one deletion mutation in exon 10 and one nonsense mutation in exon 26. Both mutations resulted in early truncation and loss of function of TRPM6. The mother contained the deletion mutation in exon 10 in one of her allele, with the other one being normal. The father contained the nonsense mutation in exon 26 in one allele, with the other one being normal. None of the two sequence alterations could be seen in 106 control chromosomes.

Conclusions: The results suggested that TRPM6 gene mutations were responsible for HSH in these patients.

PP 20
A Cross-Sectional Study of Sarcopenia in Healthy Chinese Men and Women: Reference Values, Prevalence and Association with Bone Mass
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Introduction: A cross-sectional survey was conducted in Shanghai, the eastern part of China, to evaluate the prevalence of sarcopenia in Chinese men and women and compare the results to the prevalence of other populations. We also analysed the differences in sarcopenia between Chinese men and women, and assessed the effect of lean mass and fat mass of different regions on bone mass.

Methods: A total of 1766 men and 1778 women with ages from 18 to 96 participated in this study. Bone mineral density of the spine, femur, lean mass and fat mass of several body regions were measured by dual energy X-ray absorptiometry (DXA). Class 1 and class 2 sarcopenia were defined by the ALM index (appendicular lean mass/height^2) 1 and 2 s.d. below the sex-specific means for young adults.

Results: Mean values for ALM index were 7.93 and 6.04 kg m^-2 for men and women aged from 18 to 40. The reference values for classes 1 and 2 sarcopenia were 7.01 and 6.08 kg m^-2 in men and 5.42 and 4.79 kg m^-2 in women. The prevalence of sarcopenia was 4.8% in women and 13.2% in men among people aged 70 or older, which is lower than that of Caucasian populations, but the same as that of Japanese and Koreans.
in Asia. Men demonstrated greater declines in muscle mass with aging than women partly due to the protective effect of fat mass on lean mass in women. Leg lean mass was the strongest factor in femur bone mass; however, trunk lean mass was the strongest factor in spine bone mass. **Conclusions:** Maintaining healthy weight is important for the elderly in order to avoid osteoporosis and sarcopenia.

**PP 21**

**Tibia Deformation Is Regulated by the Simulated Muscle Contraction and Is Related to Joint Contact Pressure: A Cadaveric Study**

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**Objective:** Bone deformation is believed as a crucial factor in bone mechanical adaptation. However, it is still controversial concerning which force, either muscular contractions or gravitational loading, mainly contribute to the bones loading in human lower extremity. Furthermore, there are inherent limitations of the strain gauge method that limit our understanding of bone deformation. Therefore, using a novel optical method, the purpose of this study was to determine tibia deformation pattern and amplitude induced by simulated muscle contraction and varying axial loading, respectively, in a cadaver model. The relationship between tibia deformation and joint contact pressure was studied as well.

**Methods:** Six cadaveric lower extremities, including the intact lower leg and truncated thigh, were used in this study. Tibia deformation was measured by tracking the relative movement between two marker clusters (three non-collinear retro-reflective markers on each cluster) that were affixed into the proximal and distal tibia, respectively. Simultaneously, tibiofemoral and tibial joint contact pressure were determined when different axial loading and simulated muscle contractions were exerted by a specially developed loading simulator.

**Results:** When quadriceps muscle was loaded (198 N–505 N), with respect to distal tibia, the proximal tibia bent to the posterior aspect by 0.12–0.25° for all specimens and bent to the lateral aspect by 0.06–0.21° for five of all specimens, respectively. Tibia deformation in five specimens followed the same pattern over different loading conditions. The largest tibia lateral and posterior bending occurred during co-contractions of upper leg muscles and plantar flexors, and of all leg muscles, respectively. Different axial loading failed to attenuate the amplitude of muscle-induced tibia deformation. For five of all specimens, the point of force application in the tibial joint shifted from medial to lateral, from anterior to posterior, respectively, while proximal tibia bent to the lateral and posterior aspect.

**Conclusions:** Results demonstrate that the pattern and magnitude of tibia deformation, as loaded in the present configuration, were dominated by the contraction of different muscle groups, and not so much affected by axial loading. To some extent, the joint contact pressure is able to reflect the tibia deformation. We therefore conclude that the proposed method will be able to also pick up muscle-induced bone deformation in vivo. Furthermore, an in vivo study focusing on human tibia deformation measurements is expected to be performed in July 2012. The results will be reported during the conference as soon as the data are available.

**PP 22**

**Whole-Exome Sequencing Identifies a Novel VCP Mutation as the Cause of Atypical IBMPFD in a Chinese Family**

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**Aims:** Inclusion-body myopathy (IBM) with Paget’s disease of bone (PDB) and frontotemporal dementia (FTD), designated as IBMPFD, is a rare, autosomal dominant disorder (MIM #605382). IBMPFD is caused by mutations in the gene that encodes valosin-containing protein (VCP). We investigated a Chinese family in which multiple members were diagnosed with PDB and suffered from weakness of the limbs. However, no members of this family were diagnosed with FTD. We made a preliminary diagnosis of PDB, which is typically caused by mutations in the SQSTM1 gene. However, direct Sanger sequencing failed to identify an SQSTM1 mutation in any of the patients. We used whole-exome sequencing to identify the pathogenic gene mutation affecting the Chinese male proband.

**Methods:** A total of 254 subjects were examined in this study, One 56-year-old male proband, four affected, related individuals and an additional nine family members from a non-consanguineous Chinese family participated in this study. In addition, 240 unrelated, healthy individuals were used as controls. Genomic DNA was extracted from each subject. All eight exons and the exon-intron boundaries of the SQSTM1 gene were amplified by polymerase chain reaction (PCR) and directly sequenced in five patients (II13, II4, II5, II8, II9). We also sequenced the entire exome of the male proband to identify the pathogenic gene. We identified a novel mutation in VCP as the disease-causing mutation. We confirmed the result by sequencing a 500-bp region of the promoter and the coding region of VCP in all 254 of the participants using Sanger sequencing. We were able to model the structure of the mutant VCP based on the crystal structure of the wild-type VCP protein (PDB ID 3CF3) to explore the impact of this mutation on protein function.

**Results:** No mutation in the SQSTM1 gene was identified in the five patients examined using direct Sanger sequencing. However, through whole-exome sequencing we were able to identify a novel missense mutation in exon 3 of the VCP gene (p.Gly97GlU) in the Chinese male proband. This mutation was confirmed using Sanger sequencing. The proband and
Conclusions: Our findings confirm that VCP gene mutations can be a pathogenic cause of IBMPFD. This study illustrates the advantage of using whole-exome sequencing to identify genetic mutations associated with inherited disorders, particularly in cases with an atypical clinical presentation.

Aims: Familial isolated primary hyperparathyroidism (FIHP) is an autosomal dominant disorder that either represents an incomplete variant of other familial hyperparathyroidism syndrome such as multiple endocrine neoplasia type 1 (MEN1), hyperparathyroidism–jaw tumor syndrome (HPT-JT), familial hypocalciuric hypercalcemia (FHH) or is caused by a distinct clinical entities. The mutation of CDC73 (also HRPT2) gene has a high correlation with parathyroid carcinoma (PC), atypical parathyroid adenoma and cystic change. Here we identified the CDC73 gene mutation in a provisional FIHP kindred whose proband was diagnosed with PC.

Methods: The diagnosis of FIHP was made according to clinical manifestations, biochemical tests, imaging studies and pathological examination. DNA was isolated from the subjects’ peripheral blood. All coding exons and exon–intron boundaries of the CDC73 gene were amplified by polymerase chain reaction (PCR) in the proband. The region of interest was amplified in other family members (the proband’s mother, father, aunt, maternal parents). PCR products were subjected to direct sequencing.

Results: The proband was found to have PHPT during hospitalization for the treatment of spontaneous limbs fractures at the age of 21. The parathyroid adenoma was resected and confirmed as PC. PHPT was diagnosed in his mother at the age of 40 when she was admitted for bilateral kidney stones, and a parathyroid adenoma (PA) was diagnosed after parathyroidectomy. Other family members did not show any signs of hyperparathyroidism. In the absence of clinical, biochemical and radiological evidence of MEN 1, HPT-JT or FHH, the diagnosis of FIHP was made. According to the CDC73 gene mutation analysis, we identified a novel germline heterozygous G to A substitution in intron 3 (c.307+1 G>A) in the proband of his mother and his maternal grandfather. This mutation resulted in a change at the splice site producing an aberrant splicing of mRNA that would lead to a prematurely truncated protein.

Conclusions: Through the study in the FIHP kindred with PC and PA, we found a novel splice site mutation of the CDC73 gene. Despite the reported rarity of CDC73 mutations in FIHP, a person with PC in FIHP mandates serious consideration of germline CDC73 mutation status. This genetic information can be used in diagnosis, management and follow-up considerations, leading to early detection and removal of potentially malignant parathyroid tumors.

PP 23

A Chinese Kindred of Familial Isolated Primary Hyperparathyroidism Caused by a Novel Splice Site Mutation of the CDC73 Gene

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Conclusions: Through the study in the FIHP kindred with PC and PA, we found a novel splice site mutation of the CDC73 gene. Despite the reported rarity of CDC73 mutations in FIHP, a person with PC in FIHP mandates serious consideration of germline CDC73 mutation status. This genetic information can be used in diagnosis, management and follow-up considerations, leading to early detection and removal of potentially malignant parathyroid tumors.

PP 24

The Role of RANK in Breast and Prostate Cancer Growth in a Murine Model of Bone Metastasis

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Background: Most breast and prostate cancers express RANK with high expression concordance between primary tumours and their skeletal secondaries. We previously proposed that direct cross-talk between osteoblasts and cancer cells via RANKL and IL-6 enhances the growth of cancer metastases in the bone environment. However, the role of tumor-expressed RANK within this pathway remained unknown. In the present study we determined whether knockdown of RANK in breast and prostate cancer cells affects tumor growth in bone and soft tissues.

Methods and Results: RANK expression was knocked down in breast (MDA-MB-231) and prostate (PC3) cancer cell lines. Non-target (NT) sequences served as controls. Knockdown (KD) efficacy was 80% (real-time RT–PCR and western blot). In-vitro, RANK knockdown had no effect on cell growth of either cell line. In-vivo, however, and compared to the respective NT controls, intratibial injection of MDA1234 RANK or PC31234 RANK cells resulted in significantly reduced radiographic osteolytic lesions at all time points (P<0.05). Histologic analysis of bone sections at the end point demonstrated significantly smaller total tumor areas (P<0.05), reduced cortical bone destruction (P<0.01) and fewer osteoclast-lined bone surfaces at the bone/tumor interface (P<0.05) in mice injected with RANK knockdown cells compared to controls. In addition, tumors derived from RANK knockdown cells were characterized by lower mitotic activity (P<0.01) and higher rates of apoptosis (P<0.01), compared to controls.

Growth of MDA or PC3 cells implanted orthotopically into soft tissue was independent of whether animals were injected with RANK knockdown or NT cells. Interestingly, in the intratibial models, serum RANKL levels were significantly lower (P<0.05) in animals injected with RANK knockdown cells compared to NT controls, while there was no difference between groups in the subcutaneous models.

Conclusions: Expression of RANK by breast and prostate cancer cells is an important determinant of tumor growth within the bone environment. Thus, RANK expression by tumor cells enables RANKL-expressing osteoblasts to directly communicate with the cancer cells in bone, inducing both increased...
IL-6 and RANK expression by the tumor. Targeting the components of this novel feed-forward loop may offer potential new treatment strategies to control the growth of cancer bone metastases in humans.

PP 25
Serum 25-Hydroxyvitamin D Is Associated with Insulin Resistance and Beta Cell Function in Chinese Population
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Aim: To assess the associations of the serum vitamin D level, as measured by the serum 25-hydroxyvitamin (25(OH)D) concentration, with insulin resistance and beta cell function in a Chinese population.

Methods: This cross-sectional study involved 1843 participants free of Type 2 diabetes who were randomly sampled in Shanghai. The serum 25(OH)D, fasting plasma glucose and insulin concentrations, and other biochemical parameters were determined using blood samples obtained within the winter season. Insulin resistance and beta cell function were assessed using the homeostasis model assessments of insulin resistance (HOMA-IR) and beta cell function (HOMA-B), respectively.

Results: Multiple linear regression analyses adjusted for age, PTH, Ca and BMI indicated independent inverse associations between serum 25(OH)D concentrations with both HOMA-IR (P<0.001) and HOMA-B (P=0.001) in women. In men, the serum 25(OH)D concentrations were inversely associated with HOMA-IR and HOMA-B in models adjusted for age, PTH and Ca. When further adjusted for BMI, the association remained significant for HOMA-IR (P=0.022) but not for HOMA-B (P=0.302) in men.

Conclusions: The vitamin D level was significantly and independently associated with IR in a large healthy Chinese population at risk for Type 2 diabetes. Our study clearly showed that vitamin D deficiency may play a role in the pathogenesis of diabetes not only in obese individuals but also in relatively lean individuals.

PP 26
An Osteoporosis Knowledge Assessment in Older Women of an Outpatient Clinic
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Objective: Osteoporosis is often undiagnosed and untreated worldwide, especially in China. Women have a greater risk of having osteoporosis than men. Even individuals with several risk factors, including older age, having other diseases and taking drugs that would cause osteoporosis, are not more likely to be diagnosed or treated. This study aimed to identify patient characteristics associated with osteoporosis among those older women who went to outpatient clinics for health problems.

Methods: In this cross-sectional study, an osteoporosis knowledge assessment questionnaire that covered three content areas: lifestyle, symptoms of osteoporosis and effects of osteoporosis, was used to collect data. It was administered by interview during patients’ visit to the outpatient clinic of the China–Japan friendship hospital. A patient sample (n=781) comprised of three groups of women aged 50–60 years, 61–70 years, and over 70 years was taken. Statistical analyses were performed by SPSS version 12.

Results: Respondents had a mean age of 63.4 years (range, 50–88; s.d. 8.1) and a mean weight of 59.9 kg (range, 34.6–87.4; s.d. 9.3). 11.5% of women patients had early menopause (<45 years). 5.63% of the patients had a history of low trauma, and 28.9% of the patients had a history of osteoporosis; most of these patients were in the older age group. 48.4% of the patients had taken calcium supplements, but only 15.6% had taken ViD supplements. 8.2% of the patients had a history of taking corticosteroids, but there was no correlation between steroid use and osteoporosis history. A fracture history was associated with a history of osteoporosis. Only 24.7% of patients had had a BMD test (DEXA). Of 34 patients who had low BMD (T<–2.5), 70.6% had received a calcium supplement, but only 8 patients (23.5%) had received anti-osteoporosis treatment at the time of survey. Six hundred sixteen patients received DEXA testing during the visit and 18.5% of them had osteoporosis and 56% had osteopenia.

Conclusions: Older women in outpatient clinics may have a high prevalence of osteoporosis risk factors, but are not sufficiently screened by BMD or offered treatment. Measures should be taken to improve identification of osteoporosis treatment of those patients at high risk for osteoporosis.

PP 27
Interrelationship between Trabecular Bone Architecture and Principal Mechanical Strength Prediction by 3-D Noninvasive Quantitative Ultrasound and Finite Element Analysis as a Means of Bone Quality Assessment for Osteoporosis
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Objective: Trabecular architecture is a primary parameter that contributes to bone quality and structure risk prediction. The alignment of trabecular bone is heavily influenced by the received particular functional and mechanical milieu. Our previous work has shown that quantitative ultrasound (QUS) has the ability to predict the principal structural orientation (PSO) of trabecular bone. This study aims to evaluate the interrelationship between QUS-predicted trabecular bone architecture and microCT-based meshed finite element analysis (FEA)-determined principal bone strength as a means to assess trabecular bone strength.

Methods: A total of seven trabecular bone samples, in 3-D spherical shape, were machined from distal bovine femurs. Rotational QUS scanning with an increment of 10° was performed to predict the PSO. MicroCT with a resolution of 36.9
micron was performed to obtain the geometries of the samples and the mean intercept length (MIL) tensor. The microCT images were converted into mesh models of tetrahedral elements for FEA. Young’s modulus of 18.9 GPa and Poisson’s ratio of 0.3 were assigned to the FEA models. A displacement-controlled loading of 2000 microstrain was applied to each model in six directions: antero-posterior (AP), medio-lateral (ML), proximal-distal (PD), longest vector of MIL tensor, the PSO predicted by ultrasound velocity (UV) and ultrasound attenuation (ATT).

**Results:** The stiffness of each bone sample was calculated from the reaction force of the whole bone ball; the strain and stress in the loading direction, maximum principal strain and stress, and von Mises stress were the average outcome of all the elements in a model. No statistically significant difference of these mechanical properties was found among measurements of loading in six directions. A trend can be observed that the measurements of mechanical properties in the orientations of UV, ATT and MIL are higher than the anatomical orientations. The stiffness differences between UV and MIL, and ATT and MIL are 3.6% and 1.4%, respectively.

**Conclusion:** The mechanical properties measured in the PSO predicted by QUS were highly close to the ones in the MIL orientation. These results validate the ability of QUS in predicting the PSO of trabecular bone. The promising results suggest that 3-D QUS can be used as a noninvasive modality to predict trabecular principal orientation in normal and osteoporotic bones. The propagation of QUS wave in bone structure needs further study to provide the mechanism of such measurement. This work is kindly supported by NASA/NSBRI and NYSTAR.

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**PP 28**  
**A Novel Heterozygous Mutation in SLC34A3 Gene Causes Hereditary Hypophosphatemic Rickets with Hypercalciuria**  
**Yue Chi, Weibo Xia, Yue Sun, Zhen Zhao, Yan Jiang, Mei Li, Ou Wang, Xiaoping Xing, Xueying Zhou, Kunwu Meng**  
Department of Endocrinology, Key Laboratory of Endocrinology, Ministry of Health, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing, China

**Aims:** Hereditary hypophosphatemic rickets with hypercalciuria (HHRH) is a rare metabolic disorder inherited in an autosomal recessive fashion, characterized by hypophosphatemia, short stature, rickets, and/or osteomalacia and secondary absorptive hypercalciuria. In two reports on six affected kindreds with HHRH, the disease was mapped to chromosome 9q34, which contains the SLC34A3 gene that encodes the renal type 2c sodium-phosphate cotransporter. Our aim was to screen the SLC34A3 gene of one patient with typical manifestations of HHRH to determine if there was a genetic contribution to the patient.

**Methods:** We described a 29-year-old man who had rickets as a child and had hypophosphatemia, bone pain, elevated 1,25(OH)₂D level and recurrent nephrolithiasis. Mutation analysis of exons and adjacent introns in the SLC34A3 gene of the patient was conducted.

**Results:** The genetic analysis revealed one novel heterozygous missense mutation in exon 12 c.1402C>T (p.R468W) in the SLC34A3 gene of the patient.

**Conclusions:** It has been reported that heterozygous SLC34A3 mutations lead to a variety of biochemical abnormalities. Our findings in this study suggest that the mutation in heterozygosis likely gave rise to a mild phenotype with different penetrance. The amino acid 468 of NPT2c is conserved among species (Arginine). The missense mutation likely disrupts the normal function of the transporter. Thus, this result raises some issues on the genetic basis and pathophysiological mechanism of hereditary hypophosphatemic rickets with hypercalciuria.

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**PP 29**  
**Fracture Risk Assessment and Treatment Locally Applied Simvastatin Improves Fracture Healing in Osteoporotic Rat**  
**Faming Tian**  
Medical Research Center, Hebei United University, Tangshan, China

**Aims:** Many clinical and experimental studies reported simvastatin as a potential stimulator of bone formation. In this study, the effect of simvastatin locally applied from a bioactive polymer coating of implants on osteoporotic fracture healing was investigated.

**Methods:** Thirty female 3-month old Sprague-Dawley rats underwent ovariectomy (OVX, n=20) or sham operation (n=10). Six weeks later, osteoporosis was confirmed in the OVX rats. Bone mineral density (BMD) was measured using dual-energy X-ray absorptiometry (DXA) of the left total femur. An open right femoral midshaft fracture was created and intramedullary stabilization was achieved with uncoated titanium Kirschner wires in normal rats (group A), with polymer-only coated vs. polymer plus simvastatin coated titanium Kirschner wires in osteoporotic rats (groups B and C, respectively). All rats were killed at 12 weeks post fracture, and then the right femurs were harvested for DXA Scans of bone mineral density, X-ray evaluation, histologic analysis and immunochemical staining for VEGF and BMP-2.

**Results:** (1) BMD measurement: 6 weeks after ovariectomy operation, the BMD of OVX rats was significantly lower than the Sham rats, which suggested that the animal model of osteoporosis was established successfully. In contrast to group B, significant increases in tBMD and nBMD were observed in group A and C, 12 weeks after fracture operation.

(2) Radiographic evaluation: Radiographic results demonstrated the complete healing of the fracture in group A indicated by the near disappearance of the fracture gap, while delayed healing was observed in group B for fracture gaps were still in remodelling. Rats in group C showed progressed callus formation with an obscured fracture gap compared to those in group B, though lower density were observed compared to those in group A.

(3) Histological observation: In group A, the original callus was replaced by lamellar bone while delayed fracture healing was observed in group B characterized by partial filling with cartilage callus. Group C showed more mature callus that was in the remodelling process from woven bone into lamellar bone.

(4) Immunohistochemical staining: no significant differences were observed in the expression of BMP-2 and VEGF in the fracture callus between any two groups.
Conclusions: The present study revealed improved fracture healing in response to simvastatin locally applied from a bioactive polymer coating of implants in osteoporotic rats. In terms of the results of previous studies that upregulation of BMP-2 and VEGF participate in the process of simvastatin stimulated bone formation, the unexpected results in the present study might be due to the different phase of fracture healing in each group.

PP 30
Micro-CT-Based Angiography for Studying Angiogenesis of Long Bone Fracture Repair in a Rat Model: A Comparison with Immunohistochemistry
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Objective: To explore microcomputed tomography (MicroCT) based angiography for exhibiting the neovascularization/angiogenesis of a callus in a rat femoral fracture model.

Methods: After establishment of a closed fracture, 60 male SD rats were randomized into 2 groups (30 in each group) and killed at the time points of 1, 2, 3, 4 and 8 weeks. The calluses in the experimental group were scanned by Micro-CT and 3-D vasculature images were reconstructed. Vessel size distribution, total vessel volume and volume fraction were quantified. The calluses in the control group were assessed by immunohistochemistry for the expression of vascular endothelial growth factor (VEGF) and VEGF receptor 2 (VEGFR-2).

Results: Micro-CT based angiography provided native 3-D vasculature images to reveal the states of neovascularization. The total vessel volume and volume fraction peaked at 3 weeks (196±20.33 mm³) and (6.7±0.74%), respectively (P<0.05). Immunohistochemistry of callus sections showed the expression of VEGF and VEGFR-2 occurred in the early stage of fracture healing and peaked at 2 weeks, the number of positive cells were (113.40±9.17) and (51.80±4.24) respectively (P<0.05).

Conclusion: Micro-CT based angiography atraumatically provided high-resolution, quantitative, 3-dimensional and objective data analysis. Micro-CT based angiography is a robust methodology for evaluation of vascular networks in the callus of a small animal.

PP 31
Association of Body Shape and Fracture Risk
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Objective: To examine the independent association of body shape (total volume, trunk volume, and trunk to leg volume ratio) to fracture risk.

Methods: We performed a cross-sectional study of the NHANES 1999–2004 survey data downloaded from the study website. Included individuals had both a valid dual-energy X-ray absorptiometry (DXA) scan and known fracture status. The body shape measures were derived using DXA whole body scans results and in-house algorithms. Linear regression and multivariate logistic models were created to examine the associations of total body volume, trunk volume, and the ratio of trunk to leg volume to subsequent fracture risk after controlling for other known risk factors including weight, body mass index (BMI), bone mineral density (BMD), and lean mass.

Results: 9876 of 31126 adult subjects had both valid questionnaire and DXA results. 1086 individual stated they had a previous fracture of the hip (74), wrist (891), or spine (182). Age, total BMD, total lean, total volume, trunk volume, and trunk to leg volume ratio had ranges (mean±standard deviation) from 20 to 85 years old (49.3±18.9), 0.637 to 1.742 g cm⁻² (1.123±0.128), 20.50–89.86 g (48.88±11.11), 25.1–140.2 L (76.8±16.4), 13.1–73.1 L (38.1±9.0) and 0.81–2.83 (1.53±0.24) respectively. We found all shape measures to be highly correlated with weight, BMI and total body lean (r=0.74–0.99), but weakly correlated to BMD (r=0.31–0.42). Trunk to leg volume ratio increased by age (r=0.36), but was significantly higher in men than women, and in Mexican-Americans versus other ethnicities. While total volume was associated to all fractures (odds ratio per standard deviation (OR/s.d.=0.73), only trunk volume was associated to wrist fracture (OR/s.d.=0.74), and only trunk to leg volume ratio was associated with vertebral fracture (OR/s.d.=1.24).

Conclusion: While overall large body size (total or trunk volume) was protective of all fracture, individuals with a high trunk to leg volume ratio (‘apple’ shape) have an increased risk of vertebral fractures independent of age, weight, BMI, BMD and lean mass.

PP 32
Femoral Shaft Fractures of AO Type A and B1: A Comparative Study Between Locked and Expandable Nailing
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Objective: To discuss the advantages and disadvantages of expandable and locked intramedullary nailing on treating of femoral fracture of 32A and 32B, evaluate their clinical results in order to provide some suggestions for clinical treatment, and to help choose the best treating methods.

Methods: From June 2006 to March 2011, 20 patients suffering from femoral fractures were treated surgically in our department with the method of Fixion nailing. They were all AO type 32A and 32B1. We retrospectively chose another 20 patients (group B) who had the same femoral shaft fractures from our past patients who were treated with conventional locked intramedullary nailing, and compare these following aspects: operation time, X-ray exposure, amount of blood lost, healing time, hospitalization time, time to full-weight bearing, time to achieve bony union, further surgery or significant complications such as fat embolism. They were all recorded.

Results: All the patients were followed up for 12–24 months. All the patients achieve bony union. Group A is superior to Group B on operation time, X-ray exposure, amount of blood lost, healing time, hospitalization time. Group B does not need...
to take fluoroscopy repeatedly. However, there is no significant difference on the time to full-weight bearing between Group A and Group B.

Conclusions: Compared with lock intramedullary nails, expandable intramedullary nails can reduce the operation time, X-ray exposure, amount of blood lost, healing time, and hospitalization time. We conclude that Fixion nailing is a better method than the traditional locked nailings at least in treating femoral diaphyseal fractures of 32A and 32B1. So we suggest the expandable Fixion nailing the first choice treating with fractures of 32A and 32B1.

PP 33
Cost-Effective Radiological Imaging and Processing Can Differentiate Fractured and Non-fractured Individuals in Osteoporotic and Non-Osteoporotic Individuals
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Objective: In this study we assessed clinically whether texture analysis, in comparison to bone mineral density (BMD) from quantitative-CT (QCT), can differentiate fractured and non-fractured in osteoporotic and non-osteoporotic individuals in India, where availability of QCT or DXA (dual-energy X-ray absorptiometry), for BMD assessment, is on the lower side and cost on the higher side of their respective spectrums.

Methods: Individuals who were diagnosed with fragility fractures of hip, spine and wrist were included in the study and individuals who were suspected of taking drugs related to bone strength and with known bone metabolism diseases were excluded. The study sample included 52 fractured individuals (hip=40, vertebral=12) and 14 controls, who came to the hospital for reasons other than fracture. Age profile between the two groups was similar (P>0.05). BMD measurements, using QCT, were obtained at lumbar levels L1, L2 and L3. Radiographic images were taken at the calcaneus and run-length texture parameters were obtained using a Matlab code. Individuals were divided into two samples (osteoporotic and non-osteoporotic) and statistically analysed separately.

Results: Since there were no non-fractured in the osteoporotic sample, all individuals (osteoporotic and non-osteoporotic) were included in the first analysis. Both BMD variables and texture variables were showing significant differences between groups. However, in terms of effect size, the BMD variable (T-score−1.63) had greater value compared to the texture variable (grey level non-uniformity (GLN)−1.10). But both values are considered relatively large (>0.8), if we go by general understanding of effect size. Similarly, ROC curve area of BMD variable (T-score−0.91) was superior to texture variable (GLN−0.77), but not significantly different. Notably, in non-osteoporotic sample analysis, none of the BMD variables were able to distinguish fractured from non-fractured groups. Whereas some texture variables, like run length non-uniformity (RLN), GLN, run percentage (RP) were showing significant differences between groups.

Conclusion: Similar to BMD, texture parameters were able to differentiate fractured and non-fractured individuals, especially in non-osteoporotic sample where BMD failed. However, BMD was showing overall better values in terms of effect size and discrimination of groups. In conclusion, texture analysis technique needs to be refined more and standardized for better results and clinical applicability. Because of the easy availability of radiography, cost, safety and practicality, texture technique can be very useful in conducting mass-screening, especially in developing countries like India and China, to estimate risk and avoid fractures, which can increase economic and social burden.

PP 34
Combination Of Quantitative Ultrasound and FRAX® in Evaluation of Structural–Functional State of Bone in Postmenopausal Women
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The aim of the study was to estimate the informative value of quantitative ultrasound and its combination with FRAX® in evaluation of structural-functional state of bone in Ukrainian postmenopausal women.

Materials and Methods: 363 postmenopausal women aged 45–87 years were examined, average age 65.1±0.5 years, duration of postmenopausal period 16.5±0.5 years. Bone mineral density (BMD) was measured by dual-energy X-ray absorptiometry (DXA) ‘Prodigy’ and calcaneal quantitative ultrasound (QUS) ‘Sahara’. The 10-year probability of major osteoporotic fracture was calculated with FRAX® tool.

Results: The distribution of bone indexes depended on the measurement method used. Among women who had osteoporosis of femoral neck by DXA, 34% had osteoporosis, 57% osteopenia, and 9% were normal by QUS. Sensitivity of QUS indexes ranged from low to moderate, but specificity was low (with femoral neck −38 and 39%, total hip −63 and 34%, lumbar spine −45 and 34%, total body −56 and 34% accordingly). Such sensitivity and specificity increased when combining QUS with the ten year probability of major osteoporotic fracture without BMD (FRAX®) (with femoral neck −71 and 87%, total hip −90 and 100%, lumbar spine −72 and 83%, total body −79 and 91% accordingly).

Conclusions: QUS is informative method in evaluation of structural and functional state of bone in postmenopausal women. Sensitivity and specificity increased when combining QUS with FRAX® from 38 and 34% up to 90 and 100% accordingly.

PP 35
Vitamin D status and its relationship with body composition, bone mineral density and fracture risk in postmenopausal central south Chinese women
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Aims: To assess vitamin D [25(OH)D] status and its relationship with body composition, bone mineral density (BMD), serum parathyroid level (PTH) and fracture risk, as determined using the FRAX algorithm, among postmenopausal Central South Chinese women, and to identify the risk factors for vitamin D deficiency and for osteoporosis.
Methods: This cross-sectional study involved 609 healthy postmenopausal central south Chinese (28°N) women aged 50–77 years. Total body composition and BMD at the lumbar spine (L1–L4), left femur neck and total hip were measured with dual X-ray absorptiometry, and serum 25(OH)D and PTH levels, with quantitative sandwich enzyme-linked immunosorbent assay. The 10-year probabilities of a hip fracture and major osteoporotic fracture were calculated by the FRAX model.

Results: Approximately 72.6% women were vitamin D deficient (25(OH)D $< 50$ nmol$^{-1}$). Serum 25(OH)D levels did not correlate with age at menopause, PTH levels, body mass index (BMI), fat mass and weight. They positively correlated with all BMDs ($P<0.05$) and negatively correlated with both 10-year fracture probabilities ($P<0.05$). Logistic regression also showed that smoking was risk factor for vitamin D deficiency. It also showed that BMI $\leq 19$ kg$\cdot$m$^{-2}$ and age $>65$ years were risk factors for osteoporosis of the lumbar spine (L1–L4), femoral neck and total hip, and 25(OH)D $< 50$ nmol$^{-1}$ was a risk factor for femoral neck osteoporosis.

Conclusions: Vitamin D deficiency was prevalent among postmenopausal Central South Chinese women. Smoking was a risk factor for this deficiency, and vitamin D level was associated with a decreased risk of fractures.

PP 36
FGFR3 Binds Type AKT to Facilitate its Degradation and Negatively Regulates Skeletal Development
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Multiple constitutively active mutations of FGFR3 can lead to achondroplasia (ACH), one of the most common dwarfsisms in humans, but the molecular mechanism remains elusive. In this study, we found that FGFR3 inhibiting AKT1 signaling contributes to the progress of bone development related to ACH. FGFR3 directly binds unphosphorylated AKT1, but not the constitutive activated form, to facilitate its degradation through CHIP-mediated ubiquitination pathway. Using in vitro embryonic bone culture system, we showed that BpV, a specific inhibitor of PTEN, increased the growth of cultured bone rudiment. Furthermore, we demonstrated that PTEN ablation in chondrocytes rescued the bone phenotype observed in FGFR3 knock-in mice by increasing chondrocyte proliferation and differentiation. Our findings reveal that FGF/FGFR3 plays an important role in the regulation of chondrogenesis and development of achondroplasia by boosting the degradition of unphosphorylated AKT1.

PP 37
Exogenous PTH1-34 Can Rescue the Delayed Fracture Healing Caused
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Objective: Achondroplasia (ACH) is the most common form of dwarfism and a kind of autosomal dominant inheritable disease caused by activated mutations in the coding sequence of the FGFR3 gene. The main phenotype is retardation of long bone development resulting from disturbed proliferation and differentiation of growth plate chondrocytes. As a regulator of bone growth, FGFR3 is also involved in the regulation of fracture repair. Gain-of-function mutation of FGFR3 in mice (FGFR3$^{G380C/+}$ mice, ACH mice) resulted in delayed fracture healing by inhibiting chondrocyte differentiation and bone resorption. The expression of PTHrP in the callus is lower in ACH than in Wild-type mice. There have been a variety of studies on the role of PTH1-34 in fracture healing, which demonstrated that PTH1-34 preferentially enhanced chondrocyte recruitment and the rate of chondrocyte maturation to stimulate endochondral ossification. In this study, we used a mouse model mimicking human achondroplasia caused by a gain-of-function mutation of FGFR3 to explore the effect of exogenous PTH1-34 on the delayed fracture healing resulting from ACH.

Methods: Closed fracture of the proximal tibia was created and stabilized with an intramedullary pin in 7–8-week-old mice. The mice were randomly divided into treatment and control groups. The mice in the treatment group were given PTH1-34 80 µg/(kg/d) subcutaneously until the end of the observation period while the control mice were given sterile water. Callus tissues were analyzed at 1–4 weeks post-fracture by radiography and histology. The concentrations of serum calcium and phosphorus were measured. RNA was isolated from callus tissues, and the expression levels of bone formation-related genes were evaluated by real-time PCR.

Results: At 7 days post fracture, cartilaginous callus areas were increased in PTH1-34-treated ACH mice compared with those of control mice. In contrast, at 14 days post-fracture, the remnant cartilaginous callus areas were smaller in PTH1-34-treated mice than those in control mice. There was remnant cartilaginous callus in ACH control mice while little remnant cartilaginous callus was observed at 21 days after fracture. The remnant fibrous bone areas in the marrow cavity were also reduced in PTH1-34-treated mice compared to control mice at 28 days after fracture. In the early stages of fracture (7 days), compared with the control group, PCNA and COL10A1 mRNA expression was obviously increased in the PTH1-34 treatment group. In the middle stages of fracture (14 days), compared with the control group, the OC mRNA expression level was significantly increased in PTH1-34 treatment group.

Conclusions: Exogenous PTH1-34 promoted bone fracture healing in terms of increasing callus area and endochondral ossification.

PP 38
Effects of Zoledronate on Bone Mineral Density, Indices of Bone Metabolism and the Risk of Fracture in Postmenopausal Osteoporosis Patients
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Objective: To observe the effects of Zoledronate on bone mineral density (BMD), indices of bone metabolism and the risk of fracture in postmenopausal osteoporosis (PMOP) patients.

Poster abstracts
Methods: 558 PMOP patients, with ages ranging from 55 to 64 years, were randomized to three groups. All of them received 'GAIERQI D’Capsules 600 mg per day and calcitriol 0.25 µg per day; Group A, consisting of 185 patients, received Zoledronate 4 mg per day; Group B, consisting of 183 patients, received Livial 2.5 mg/day; the rest received ‘GAIERQI D’Capsules and calcitriol only. Dual-energy X-ray absorptiometry (DEXA) and measurements of a series of biochemical indices were performed before and after medication at 6, 12, 24 and 36 months.

Results: BMD increased in the Zoledronate and Livial groups, especially in the lumbar spine. In the Livial group, the levels of E2 increased rapidly (P=0.0012) and levels of CTX decreased (P=0.0025). In the Zoledronate group, no changes in levels of E2 (P>0.05) occurred, levels of BALP and OC increased (P<0.05) and levels of CTX decreased (P<0.05). The risk of low trauma fracture decreased in the Zoledronate (P=0.0052) and Livial groups (P=0.0061).

Conclusion: Zoledronate significantly improves BMD and reduces the risk of fracture to a similar degree as Livial. Thus, it may play an important role in the treatment of postmenopausal osteoporosis. Zoledronate can improve the compliance and safety of patients. Calcium tablets did not prevent bone loss.

PP 39
Bone Healing Enhancement through Inhibition of Sclerostin by Monoclonal Antibody in Rat Osteotomy Model
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Aim: Sclerostin is a negative regulator of bone formation. Previous studies demonstrated that treatment with a sclerostin monoclonal antibody (Scl-Ab) significantly increased bone formation, bone mass and strength in a rat close fracture model. The aim of this study is to investigate the effects of systemic administration of Scl-Ab on fracture repair in rat femur open fracture, a more difficult-to-heal model.

Methods: Ninety 6-month-old male SD rats were randomly divided into Scl-Ab and vehicle groups following a transverse osteotomy at the mid-shaft of the right femur. One day post surgery, rats were treated with Scl-Ab III (s.c. injection, 25 mg/kg) or vehicle. Rats were sacrificed at weeks 3 and at week 6. Hematoxylin and eosin (H&E) staining and safranin O staining showed more bony tissue in calluses at week 3 in the Scl-Ab group. Four-point bending test revealed significantly higher ultimate load in the Scl-Ab group than in the vehicle group at weeks 6 (+98%, P<0.01) and 9 (+45%, P<0.05) post fracture. In addition, ultimate load at week 6 in the Scl-Ab group reached a similar level as seen at week 9 in the vehicle group, indicating the faster biomechanical healing by Scl-Ab in this model. Stiffness and energy to failure also tended to be higher in the Scl-Ab group.

Conclusions: This study demonstrated that Scl-Ab enhanced bone healing in a rat osteotomy model, with increased bone formation, bone mass and bone strength. We observed a trend of increasing callus vascularity associated with Scl-Ab administration at week 3 and at week 6, suggesting that Scl-Ab might induce the coupling of osteogenesis and angiogenesis. Collectively, our results support the hypothesis that systemic administration of Scl-Ab enhances open fracture healing.

PP 40
Analysis of COL1A1 and COL1A2 Mutations in 14 Chinese Families with Osteogenesis Imperfecta
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Aim: Osteogenesis imperfecta (OI) is a heritable bone disorder manifested by brittle bones and low bone mass. OI was associated with abnormalities in the synthesis or structure of type I collagen. Approximately 90% of patients with OI have mutations in COL1A1 or COL1A2 gene. Cells harbouring such a mutation produce a mixture of normal and abnormal collagen.

Methods: Fourteen unrelated probands from 14 families were recruited from China since 2010. In the present study, mutation analyses were performed for COL1A1, COL1A2 genes in a total of 47 DNA samples from the 14 probands, 33 family members. DNA sequencing of polymerase chain reaction (PCR)-amplified COL1A1, COL1A2 gene fragments covering the entire coding region and sequencing of the intron–exon boundaries were carried out using an ABI 3730 automated sequencer and the Big Dye Terminator Sequencing protocol (ABI). In addition, control alleles from 250 normal individuals were sequenced to determine whether novel mutations occurred as polymorphisms.

Results: In our study, there were 9 male and 5 female patients whose mean age was 11.7 years. Nine patients were clinically diagnosed as OI type I, two patients as type III and 3 patients as type IV. A total of 10 patients had mutations in COL1A1 gene while 4 had mutations in COL1A2. The mutations in 14 probands included 9 missense mutations, 1 insertion mutation, 3 splicing variants and 1 nonsense mutation. Five probands could be diagnosed as haplo-insufficiency mutations and 9 patients with helical mutations. Altogether, 4 of the mutations were novel: 3 in COL1A1 and 1 in COL1A2, including c.433_434insC, c.2867G>C and c.3893C>T in COL1A1 and c.2081G>A in COL1A2.

Conclusion: These findings suggest that mutations in COL1A1 are more common than in COL1A2 in Chinese OI patients. The novel mutations c.433_434insC, c.2867G>C and c.3893C>T
in COL1A1 and c.2081G>A in COL1A2 are responsible for OI in some Chinese patients.

PP 42
The Analysis of Serum 25-Hydroxy Vitamin D in 382 Elderly Men
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Objective: To observe serum 25-hydroxy vitamin D levels in 382 elderly men.

Methods: 382 elderly men taking physical examination in April 2011 in our hospital were selected. The subjects were classified into four groups according to their ages and the serum 25OHD levels were measured by electro-chemiluminescence, followed by statistical analysis.

Results: The average level of serum 25OHD is 69.83 nmol/l; the lowest level of serum 25OHD is in the 86–90-year age group; the rest of the elderly men in general showed insufficiency except for the age group of 70–75 years according to the reference range. The rate of deficiency, insufficiency and sufficiency of 25OHD levels in the four groups is different (P<0.05).

Conclusions: Vitamin D deficiency and insufficiency are very popular in the 382 elderly men in Guangzhou. Positive propaganda and education is very urgent.

PP 43
Diversity of Clinical Features in Type V Osteogenesis Imperfecta
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Objective: Osteogenesis imperfecta (OI) is a heritable connective disorder characterized by increased bone fragility and short stature. OI type V was presumed to be an autosomal dominant inheritance without COL1A1 and COL1A2 gene mutations. OI type V has distinct clinical features. Here we summarized the characteristics of 11 Chinese patients with OI type V.

Methods: We identified 11 patients carrying typical features of OI type V. Seven patients were sporadic cases, two patients were father and daughter, the other two patients were mother and son. Six of them were male and five were female. Patients’ age ranged from 7 to 32 years. Fracture histories were investigated. Deformity of the spine and extremities, dental status, color of the sclera, body weight and height were recorded.

Results: In all 11 patients, a 13-year-old girl had blue sclera. Dentiogenesis imperfecta was absent in all patients. Number of fractures in a lifetime ranged from 0 to >20 times. Average fracture frequency was 0.92 per year. The body weight and height were normal or slightly lower than normal. All patients had ossification of the interosseous membrane of the forearm except for the daughter and father with hypertrophic callus. Heterotopic ossification around the hip joints and forearm was observed in a 22-year-old female. The radial head dislocations were present in 7 patients. Hypertrophic callus was observed in four patients according to X-ray record and five patients had scoliosis.

Conclusion: We report a typical type V OI patient with blue sclera. Hypertrophic callus or ossification of the interosseous membrane was observed in all type V patients. Heterogeneous morphologic phenotype and radiological diversity existed in OI type V. Further histological analysis and gene detection of collagen I may give further understanding of this OI type.

PP 44
Bone Mineral Density Changes and Variables Associated in Hemophilia A Patients
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Aims: To evaluate the changes of bone mineral density (BMD) in hemophilia A (HA) patients in China, and to explore the risk factors associated with BMD changes.

Methods: Forty-eight HA patients without inhibitor were enrolled in this study. Each patient completed the clinical evaluation. BMD was assessed using dual-energy X-ray absorptiometry (DXA) for all subjects. Serum samples from 39 HA patients were taken for bone turnover marker detection. Carboxy-terminal cross-linked telopeptide of type I collagen (CTX) and amino-terminal propeptide of type I pro-collagen (P1NP) were measured using electrochemiluminescence immunoassay (ECLIA).

Results: (1) In all 48 patients, the average of bilateral hip BMD Z-scores was negative. There was no significant difference in BMD between severe and moderate hemophilia patients at all measured sites. (2) In the on-demand treatment group, patients with Z-score above -2 had a higher physical activity level compared with those with score below -2 (OR 10.652, 95% CI 1.456–77.92), and a lower incidence of long-term immobility history (OR 0.057, 95% CI 0.003–0.972). (3) Patients assigned to the secondary prophylaxis regimen experienced a decrease in annual bleeding episodes and an increase in physical activity levels. In this group, physical activity levels were significantly higher in the Z-score >-2 group than those in the Z-score ≤-2 group, whether they were still on on-demand treatment (3.4±0.5 vs 2.4±0.9; P=0.029) or switched to prophylaxis (4.0±0.0 vs 3.1±0.8; P=0.009). (4) In all 39 patients, serum β-CTX was found to have a significant negative correlation to BMD Z-scores (r=-0.409; P=0.010), while there was no significant correlation found between P1NP and BMD.

Conclusions: (1) The average bilateral hip DXA measurements were well below those of the ethnic-, age- and sex-compared population. No significant difference in BMD was observed between severe and moderate hemophilia patients. (2) For those on on-demand treatment, physical activity level appeared to be a protective factor, while a history of long-term immobility proved to be a risk factor. For those on secondary prophylaxis, physical activity levels before and after prophylaxis were significantly related to BMD. (3) Secondary
prophylaxis alone was not proved to be a protective factor for the BMD of hemophilia patients. (4) Increase of bone resorption might be responsible for BMD decrease in hemophilia patients.

PP 45
The Influence of Osteoporosis on Lumbar Spinal Fusion
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Objective: To explore the influence of osteoporosis on lumbar spinal fusion.
Methods: From January 2007 to June 2011, 98 patients with lumbar disc degenerative diseases who underwent posterior lumbar spinal fusion were analyzed. There were 43 osteoporotic patients, including 10 males and 33 females with an average age of 62.09±1.22 years, and 55 patients with normal bone mass, including 31 males and 24 females with an average age of 60.38±1.14 years. All patients underwent posterior lumbar discectomy, decompression, intervertebral and/or posterolateral bone graft fusion and pedicle screw fixation. The clinical efficacy and fusion rate between two groups were compared.
Results: All patients were followed from 6 to 47 months. The preoperative data and surgical data of two groups showed no statistical difference. The ODI scores of the last follow-up were significantly improved in both groups (P<0.05). Significant difference was observed regarding the excellent and good rate between two groups, with 89.1% for the control group and 72.1% for the osteoporosis group (P<0.05). Solid fusion was achieved in 94.5% (52/55) of the patients in the control group and 79.1% (34/43) of those in the osteoporosis group (P<0.05). Postoperative complications occurred in 9 patients, with 4 cases in the control group (7.3%) and 5 cases in the osteoporosis group (11.6%), showing no statistically significant difference (P>0.05).
Conclusion: Osteoporosis is one of the key factors affecting the outcome of lumbar spinal fusion in elderly patients. Perioperative and long-term postoperative anti-osteoporosis therapy should be emphasized.

PP 46
Relationship Between Lipid Metabolism and Bone Mineral Density in Middle and Senior Men
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Objective: To explore the relationship between lipid metabolism and bone mineral density (BMD) in middle and senior men.
Methods: 529 middle and senior men were divided into three groups: age ranges of 50–59, 60–69, 70–79. BMD of the lumbar (L2–4) and proximal femur (NeckTrocH Ward's) were measured by dual-energy X-ray. Serum lipid profiles of TG, TC, LDL-ch, HDL-ch and LP(a) were examined. The results were compared among the three groups. The relationship between BMD and lipid levels was analyzed using simple linear correlation.

Results: BMDs of L2–4, Neck, Troch and Ward’s were significantly lower in the 60–69 years age group and 70–79 years age group than in the 50–59 years age group (P<0.05). BMD of L2–4 and Neck were significantly lower in the 70–79 years age group than in the 60–69 years age group (P<0.05). The levels of LDL and LP(a) were higher in the 60–69 years age group and 70–79 years age group than in the 50–59 years age group (P<0.05). The levels of HDL were lower in the 60–69 years age group and 70–79 years age group than in the 50–59 years age group (P<0.05). The levels of LP(a) were higher in the 70–79 years age group than in the 60–69 years age group (P<0.05). BMDs were related negatively to LDL and LP(a) in the three groups (P<0.05).

Conclusion: In middle and senior men, BMDs were decreased with age. BMDs were related negatively to LDL and LP(a). Regulation of lipid levels is beneficial to prevent osteoporosis.

PP 47
Bone Remodeling after PIP Joint Arthroplasty Using Pyrocarbon, a Material Isoelastic to Bone
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Objective: In PIP joint osteoarthritis, contracture and pain often develops. In 2001 the Ascension® Pyrocarbon PIP-prosthesis was introduced. Pyrocarbon is a form of carbon, durable, tissue compatible and wear-resistant. Pyrocarbon has an elastic modulus similar to cortical bone, thus minimizing the shear in the interface and relative motion between the prosthesis and surrounding bone in loading and bending/shear.
Methods: Thirty-four joints in 24 patients were operated between November 2001 and October 2005. All patients have been followed prospectively at 1, 2 and 5 years. Range of motion (ROM) and grip strength and subjective scores were recorded at 1, 2 and 5 years. Radiographically, the prosthesis position and the osseous reaction around the prosthesis were recorded.
Osseous reaction: A new scoring system is proposed to evaluate the mechanobiologic integration. The osseous reaction to the load and the stability of the inserted prosthesis were categorized into four grades.
Results: Three patients were revised. In the 21 non-revised patients, pain at rest and at activity (VAS) improved from preop to 5 years, whereas ROM and grip strength were unchanged. Migration and osseous reaction: Four proximal and three distal prostheses subsided more than 1 mm (1–2 mm). No migration occurred between years 2 and 5. Gradually, an osseous condensation developed (Figure 2) around the implant as a reaction to a functioning load transformation from the joint surface via the stem and to the endocortex. Also, in the majority of the prostheses initially showing zones of osteolysis or loosening appeared to stabilize in time. Two proximal and two distal components had osteolytic zones at 5 years.
Conclusion: Although ROM and grip strength remained unchanged, the clinical results were excellent, with 20/21 non-revised patients being pain free at rest with VAS 0 at the 5-year FU. A joint prosthesis made of pyrocarbon is isoeelastic to cortical bone and behaves differently from an un cemented or cemented stiffer metal prosthesis. A true aseptic loosening is less likely as the prosthesis and surrounding bone deform...
in concert, minimizing the relative motion in the interface. Instead, a slow remodeling of the surrounding bone takes place as a reaction to the applied forces. We propose a new grading system for the osseous reaction around a pyrocarbon prosthesis.

PP 48
Functional Analysis of Leukemia Inhibitory Factor in Osteoblast Differentiation of Bone Marrow Stromal Cells
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Objectives: Leukemia inhibitory factor (LIF) belongs to IL-6 family cytokine. Though it has been known that IL-6 induces osteoblast differentiation, the effect of LIF on murine bone marrow stromal cells (BMSCs) is unclear. The purpose of this study is to clarify the effect of LIF on osteoblast differentiation of murine BMSCs.

Methods: BMSCs were obtained from the femurs and tibiae of C57BL/6J mice, and seeded in a-MEM supplemented with antibiotics and 10% fetal bovine serum. At 3 days after seeding, non-adherent cells were removed by washing 3 times with PBS. Approximately 2 weeks after seeding, when the adherent cells had expanded to ~80% subconfluence, they were detached with trypsin-EDTA solution (0.2% trypsin, 1 mm EDTA). BMSCs were cultured in osteogenic induction medium with or without LIF for 3 weeks. At 3 weeks, cells were double-stained for alkaline phosphatase (ALP) activity and mineral deposition (von Kossa). Colony-forming units-osteoblast (CFU-O), defined as colonies with ALP-positive cells associated with mineralized matrix (von-Kossa-positive), were counted. mRNA was extracted and cDNA synthesized to determine the expression levels of ALP, Col1a, BSP, OCN, Runx2 and Osx with real-time PCR. The phosphorylation level of STAT3 induced by the stimulation of LIF was detected by western blotting.

Results: The number of CFU-Os of BMSCs cultured with osteogenic induction medium and LIF was significantly lower than that of BMSCs cultured with only osteogenic induction medium. The suppressive effect of LIF was also confirmed by the lower expression levels of osteoblast differentiation markers: ALP, Col1a, BSP, OCN, Runx2 and Osx. STAT3 was phosphorylated from 5 to 30 min after LIF stimulation. Thus, these data imply that the suppressive effect of LIF is transmitted through the LIF-STAT3 signaling pathway.

Conclusion: LIF suppresses osteoblast differentiation of murine BMSCs through the LIF-STAT3 signaling pathway.

PP 49
High-Cholesterol Diet Increases Osteoporosis Risk via Inhibiting Bone Formation in Rats
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Aim: To investigate the effects of high-cholesterol diet on the development of osteoporosis and the underlying mechanisms in rats.

Methods: Female Sprague–Dawley rats were randomly separated into three groups: (1) the high-cholesterol-fed rats were fed a high-cholesterol diet containing 77% normal diet food, 3% cholesterol and 20% lard for 3 months; (2) ovariectomised (OVX) rats were bilaterally ovariectomised and fed a standard diet; and (3) the control rats were fed the standard diet. Bone mineral density (BMD) of the rats was measured using dual-energy X-ray absorptiometry. Serum levels of oestriadiol (E2), osteocalcin (BGP) and carboxy-terminal collagen crosslinks (CTX) were measured using ELISA. Gene expression profile was determined with microarray. Mouse osteoblast cells (MC3T3-E1) were used for in vitro study. Proliferation, differentiation and oxidative stress of the osteoblasts were investigated using MTT, qRT–PCR and biochemical methods.

Results: In high-cholesterol-fed rats, the femur BMD and serum BGP level were significantly reduced, while the CTX level was significantly increased. DNA microarray analysis showed that 2290 genes were downregulated and 992 genes were upregulated in this group of rats. Of these genes, 1626 were also downregulated and 1466 were upregulated in OVX rats. In total, 370 genes were upregulated in both groups, and 976 genes were downregulated. Some of the downregulated genes were found to code for proteins involved in the transforming growth factor beta (TGF-β)/bone morphogenic protein (BMP) and Wnt signaling pathways. The upregulated genes were found to code for IL-6 and Ager with bone-resorption functions. Treatment of MC3T3-E1 cells with cholesterol (12.5–50 μg/ml) inhibited the cell proliferation and differentiation in vitro in a concentration-dependent manner. The treatment also concentration dependently reduced the expression of BMP2 and Cbfa1, and increased the oxidative injury in MC3T3-E1 cells.

Conclusion: The results suggest a close correlation between hypercholestaemia and osteoporosis. High-cholesterol diet increases the risk of osteoporosis, possible via inhibiting the differentiation and proliferation of osteoblasts.

PP 50
The Role of BMPRIA in Regulating the Differentiation of Mesenchymal Stem Cells into Osteoblasts
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Backgrounds and Objective: Osteoblasts originate from bone marrow-derived mesenchymal stem cells (BM-MSCs);
however, the mechanisms regulating the differentiation of BM-MSCs into osteoblasts remain controversial. In this study, we aimed to observe the role of type Ia receptor in the function of bone morphogenetic protein (BMPRIA) in regulating the differentiation of BM-MSCs into osteoblasts and to explore the possible involving signaling pathway.

Methods: BMPRIAs were conditionally knocked out in 3.2 kb Col 1-CreERT<sup>TM1</sup>/BMPRIA<sup>fx/+</sup> mice by Tamoxifen injection, and then X-ray, microCT, real-time PCR, HE staining, Trap staining, TUNNEL staining and immunohistochemistry (IHC) were employed to observe changes after BMPRIA knockout. β-Catenin knockout mice and dentin matrix protein (DMP1) transgenic mice were crossed with BMPRIA knockout mice to see whether they can rescue the phenotype in BMPRIA knockout mice.

Results: Bone volume increased in BMPRIA knockout mice as confirmed by HE staining and microCT. The number of RunX2 and osterix-positive cells increased in long bone, and the thickness and cell number in periosteum and endosteum increased in BMPRIA knockout mice as confirmed by IHC and HE staining. There was no significant difference between BMPRIA knockout mice and wild type mice as far as the proliferation activity of osteoblasts, the apoptosis rate of osteoblasts and the number of osteoclasts were concerned. Real-time PCR and IHC examination revealed that the expression levels of RunX2, osteocalcin, β-catenin, bone sialoprotein and ALP were upregulated in BMPRIA knockout mice, while the expression level of DMP1 was downregulated in BMPRIA knockout mice. Increased bone volume and increased number of osteoblasts were partially reversed in BMPRIA KO mice by β-catenin knockout (BMPRIA and β-catenin double-knockout mice), while DPM1 transgenic mice did not have such effects.

Conclusions: BMPRIAs negatively regulate the differentiation of BM-MSCs into osteoblasts, and BMPRIA knockout leads to increased number of osteoblasts and bone volume. β-Catenin is one of the downstream action factors of BMPRIA.
vaspin upregulated the expression of Bcl-2 protein and down-regulated that of Bax protein in a dose-dependent manner. Further study revealed that vaspin activated the phosphorylation of ERK, and pretreatment of hOBs with PD98059 (an ERK inhibitor) abolished vaspin-induced activation of ERK, while vaspin has no activation on the phosphorylation of p38, JNK or Akt in hOBs. Our results suggest that vaspin suppresses serum deprivation-induced apoptosis of hOBs through the MAPK/ERK signal pathway.

**Conclusion:** Vaspin may protect hOBs from serum-deprivation-induced apoptosis by activating the MAPK/ERK signal pathway.

**PP 54**

**Connective Tissue Growth FactorInduces Osteogenic Differentiation of Vascular Smooth Muscle Cells by ERK Signaling**

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**Aims:** Vascular calcification is an active and cell-regulated process resembling skeletal mineralization and is prevalent in patients with atherosclerosis, aging end-stage renal failure, uremia and type II diabetes. Osteogenic differentiation of vascular smooth muscle cells (VSMCs) is essential in the development of vascular calcification. Connective tissue growth factor (CTGF) plays an important role in the pathogenesis of atherosclerosis. However, the effects of CTGF on the phenotypic transformation of VSMCs and the calcification of VSMCs have not been investigated. In the present study we defined the effect of CTGF in VSMC trans-differentiation, VSMC calcification and molecular signaling by using an in vitro calcification model.

**Methods:** VSMCs used in the present studies were identified by immunohistochemistry. Cells were divided into two groups: CTGF-treated group (50ngml−1) and control group. Mineralized matrix staining was performed with 0.1% Alizarin red; the cellular mineral deposition was measured by testing the concentration of calcium. Real-time PCR and western blot were used to examine the expressions of bone markers including Cbfa-1/Runx-2, ALP, OC and OPG, and the expression levels of MAPKs, including c-jun N-terminal kinase (JNK), p38 and ERK1/2. The ERK-specific inhibitor PD98059 was used to block the activation of ERK.

**Results:** VSMCs were obtained from the thoracic aortas of mouse, and the expression of smooth muscle-specific α-smooth muscle actin antibody (α-SMA) was positive. After culture with CTGF for 14 days, the expression of bone markers including Cbfa-1/Runx-2, ALP, OC and OPG increased when compared with the control group, in a dose-dependent manner. The calcium deposition and calcium extent were also increased in VSMCs with CTGF. Western blot analysis reveals that CTGF activated ERK and the peak activation of ERK occurred at 30 min. However, CTGF has no effect on the activation of JNK and p-38. Furthermore, a ERK-specific inhibitor, PD98059, significantly suppressed the effect of CTGF on VSMC calcification and phenotypic marker expression.

**Conclusions:** Taken together, our results reveal that CTGF enhances in vitro calcification by inducing osteogenic differentiation and calcification of VSMCs, that this effect might be activated by the ERK pathway, and that ERK-specific inhibitor (PD98059) can suppress nodule formation, calcium deposition and the expression of bone markers. Our findings showed the function of CTGF in vascular calcification and may provide information in designing therapies to prevent and treat diseases relating to vascular calcification.

**PP 53**

**Association Study of Polymorphisms in the SOST Gene and Marker of Bone Metabolism in Chinese women**

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The onset and development of osteoporosis are associated with several genetic and environmental factors, including the sclerosteosis (SOST) gene. SOST is a major regulator in the canonical wingless integration (Wnt) signal pathway. This gene is mainly expressed by osteocytes and negatively regulates bone formation. It blocks Wnt signalling by binding to the Wnt co-receptors, low-density lipoprotein receptor (LRP)-5 and 6 in both osteocytes and osteoblasts. Recent study suggests that sclerostin can also inhibit BMP signaling. The aim of this study is to investigate the association of polymorphism in the SOST gene and markers of bone metabolism in Chinese women.

Seven hundred and fifty healthy females of Han ethnicity (aged 66.1±9.9 years, from 24 to 94 years) were recruited from the community centers. Ten tagging single-nucleotide polymorphisms (SNPs) of SOST (rs1234612, rs1513670, rs1634330, rs1708635, rs2023794, rs7220711, rs74252774, rs851057, rs851058 and rs865429) were identified. Markers of serum SOST, 25(OH)D, intact parathyroid hormone (PTH), β-Cross-Laps of type I collagen containing cross-linked C-telopeptide (β-CTX), calcium (Ca), phosphorus (P), alkaline phosphatase (ALP) and renal function were measured. For each SNP, a linear regression analysis with the genotype and the markers of bone metabolism showed that rs1708635, rs1634330 and rs7220711 showed significant association with β-CTX (all P<0.05); rs851058 showed significant association with serum calcium and SOST (all P<0.05). But after adjustment for Bonferroni multiple-significance-test correction, only rs1708635 and rs1634330 showed significant association with β-CTX (all P<0.005); rs851058 showed significant association with serum calcium and SOST (all P<0.005). However, after adjusting for age and BMI, the results showed no significant differences among the different genotypes of rs1708635, rs1634330 and rs851058 using analysis of covariance. These findings suggest, in this population, that common allelic variations in the SOST gene do not contribute significantly to the regulation of markers of bone metabolism.
PP 55
Macro-Structural Effect of Metal Surfaces Treated Using Computer-Assisted Yttrium-Aluminum-Garnet Laser Scanning on Bone-Implant Fixation
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Porous coatings have been applied to the surface of prosthetic devices to foster stable device fixation. The coating serves as a source of mechanical interlocking and may stimulate healthy bone growth through osseointegrated load transfer in cementless arthroplasty. Joint arthroplasty by porous-coated prostheses is one of the most common surgical treatments, and has provided painless and successful joint mobility. However, long-term success is often impaired by the loss of fixation between the prosthesis and bone. Porous-coated prostheses are associated with several disadvantages, including metal debris from porous coatings (third-body wear particles) and irregular micro-texture of metal surfaces. Consequently, quantitative histological analysis has been very difficult. These issues arise because the porous coating treatment is based on addition of material and is not precisely controllable. We recently developed a precisely controllable porous texture technique based on material removal by using yttrium-aluminum-garnet laser. Free shapes can be applied to complex, three-dimensional hard metal surfaces using this technique. In this study, tartan check shapes made by crossing grooves and dot shapes made by forming holes were produced on titanium (Ti6A14V) or cobalt chrome (CoCr) and evaluated with computer-assisted histological analysis and measurement of bone-metal interface shear strength. The width of grooves or holes ranged from 100 to 800 µm (100, 200, 500 and 800 µm), with a depth of 500 µm. While the cylindrical porous-texture-treated metal samples (diameter, 5 mm; height, 15 mm) were implanted into a rabbit femoral condyle, bone tissue with bone trabeculae formed in the grooves and holes after 2 or 4 weeks, especially in 500-µm-wide grooves. Abundant osteoconduction was consistently observed throughout 500-µm-wide grooves in both Ti6A14V and CoCr. The speed of osteoconduction was faster in Ti6A14V than in CoCr, especially in the tartan check shape made of 500-µm-wide grooves. In pushout testing, the tartan check shape made of 500-µm-wide grooves had significantly higher bone-metal interface shear strength than the dot shape or commercial porous coating. These results indicate that the tartan check shape made of 500-µm-wide grooves on metal surfaces has the potential for clinical application in artificial prosthesis design.

PP 56
Prediction and Identification of miRNAs Related to Osteoblast Function in Modeled Microgravity
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Aims: microRNAs (miRNAs) can play an important role in regulating gene expression by leading to translational restraint or mRNA degradation. Some researches demonstrate that approximately one-third of miRNAs are located within the intronic regions of coding transcription units and largely coincides with the transcription of the corresponding host genes. Microgravity modeled by a rotating wall vessel system (RWV) can induce significant changes of miRNA expression in osteoblasts. This suggests that modeled microgravity may affect the metabolism and function of osteoblasts through the alterations of miRNA expression.

Methods: We collected and analyzed those miRNAs whose expression levels were identified to be dramatically changed by microarray in mouse osteoblasts undergoing RWV-modeled microgravity and then checked their location information on chromosomes. Mirbase database was queried to look for such target miRNAs as located in the same transcription units with previously analyzed miRNAs according to location information. MC3T3-E1 cells exposed to the RWV for 48 h at 30 r.p.m. were used to confirm previously predicted miRNAs through quantitative RT–PCR (qRT–PCR) detection compared to static 1g controls.

Results: Several raw intronic miRNAs were found, including mmu-mir-335, mmu-mir-33, mmu-mir-511 and mmu-mir-5114. Target gene prediction, GO annotation clustering and KEGG pathway enrichment analysis were done to elucidate the possible roles that the five intronic miRNAs played in the functional alterations of osteoblast. Then three of them were selected randomly to perform qRT–PCR. miR-5114 shows no distinct variation between the control group and RWV group, while miR-335–3P had a slight reduction at 1.5-fold level in the RWV group and the miR-511-5P was identified to be ~2.6-fold upregulated in the RWV group (P<0.05).

Conclusions: Five potent intronic miRNAs relating to osteoblast functional alteration in modeled microgravity were predicted by bioinformatics and two of them were further confirmed using qRT–PCR. The regulatory mechanism of these potential miRNAs and their roles in the functional and morphological alteration of osteoblasts induced by modeled microgravity need to be further studied.

PP 57
Alteration in microRNA Expression of Femurs after Three Weeks of Hindlimb Unweighting in Rat
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Aims: microRNAs (miRNAs) are a growing class of small (~22 nucleotides), single-stranded noncoding RNAs found in diverse organisms that can interfere with translation of
specific target miRNAs and play an important role in regulating cellular processes. MicroRNA studies suggest a new orientation to elucidate the mechanism of bone metabolism. Recent studies have demonstrated that artificial overexpression or functional inhibition of some miRNAs, such as miR-2861, miR-23a, miR-133 and miR-141 in osteogenic lineage cells, can significantly affect their proliferation, differentiation, function and apoptosis. The positive regulation of RANKL-induced osteoclastogenesis by miR-21 has also been confirmed. To test the hypothesis that the miRNA expression of bone tissue would be altered in simulated microgravity and would result in metabolic or functional changes of bone, we studied the miRNA microarray profile of rat femurs from tail-suspended rats and the normal rats, respectively.

Methods: Twelve male Sprague–Dawley rats were randomly assigned to either the control group or hindlimb unweighting group. At the end of hindlimb unweighting for 3 weeks, rats in both groups were euthanized and femurs were harvested as soon as possible. After a quickly soft tissue elimination and bone marrow washing, the total RNAs were isolated and used for microarray profile. Part of the data obtained were confirmed by real-time PCR.

Results: Among 678 miRNAs probes evaluated, 11 were significantly upregulated and 14 were downregulated in the femurs of hindlimb unweighting rats compared with that of the control group. All of these 25 miRNAs, excepting miR-20 and miR-34, have not been reported as regulatory factors in osteoblasts. Among the significantly changed ones, four miRNAs with twofold difference were integrated into GeneGo MetaCore pathway analysis software to analyze the gene ontology, pathway distribution and putative gene targets. Bioinformatics analysis shows that the biological behavior of these four miRNAs mainly focuses on regulation of cell differentiation, apoptosis, migration, chemotaxis, ion transport and tube development, and they are also involved in such pathways as regulation of actin cytoskeleton, the Wnt signaling pathway and focal adhesion.

Conclusions: Four miRNAs relating to osteoblast functional alteration in simulated microgravity were found. The roles of these miRNAs in bone metabolism and bone loss in microgravity need to be further studied.

PP 58
Microarray Profile of Differentially Expressed Genes in Primary Osteoblasts Derived from Osteogenesis Imperfecta Patients
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Objective: Osteogenesis imperfecta (OI) is a heritable connective disorder mainly characterized by increased bone fragility and short stature. Till now, twelve clinical subtypes of OI have been described based on the clinical, biochemical and molecular nature. To identify genes involved in the process of this heterozygous disease, the differently expressed pattern of osteoblast cells was profiled by microarrays.

Methods: Primary cultures of osteoblasts were prepared from sequential enzymatic digestion of bone tissues obtained from OI patients corrected by multistepotomy and fixation with intramedullary nails. The identification of derived cells was done by histochemical staining of alkaline phosphatase (ALP) and immunohistochemical staining of type I collagen, bone sialoprotein and osteopontin. Totally, 18 primary osteoblast cells derived from OI patients and 3 osteoblasts obtained from developmental dislocation of the hip (DDH) patients were used to extract total RNA for microarray analysis. Microarray results were further tested by real-time PCR analysis. Pathway and GO analysis were performed to reveal the biological functions of these differently expressed genes.

Results: Of the approximately 40 000 cDNAs represented on the microarray, a total of 508 upregulated and 346 downregulated genes were identified with twofold exchange. To confirm the differential expression, three upregulated genes (including RIP-1, DNAJ and cyclophilin C) and three downregulated genes (IRAK2, RelB and Notch4) were selected for real-time PCR. The relative RNA expression level of these selected genes obtained by real-time PCR was similar to those obtained by microarrays. The most significant GO terms are actinin binding, vitamin transporter, heparan sulfate sulfotransferase and sis–trans isomerase. Several osteo/immune-related KEGG pathways, including MAPK, NK–kB, Toll-like receptor, inflammatory cytokines and lipid storage, were significantly overrepresented.

Conclusion: The study provides a large-scale profile of gene expression in the OI patients’ primary osteoblasts cultured in vitro. The most significant GO terms discovered in this study may play an important role in the pathology of OI. KEGG pathway analysis showed that immune response may be associated with OI, including lipid metabolism.

PP 59
Experimental Liver Cirrhosis and Bone Deterioration in Rats
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Objective: Osteoporosis (OP) has been recognized as an important complication of liver cirrhosis (LC). Nevertheless, the mechanisms by which LC causes substantial bone loss are not well understood. In this study, experimental LC disease in rats was induced by carbon tetrachloride (CCI4) administration combined with our modified diet intervention. On this basis, the LC and LC-associated bone disorders were then characterized.

Methods: Twenty-five male Sprague-Dawley (SD) rats were used in this study. In group A (n=15), LC disease was induced by our combination method. In group B (n=10), rats receiving normal feeding and natural sodium injection served as controls. Two months after modeling, the serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were detected by enzyme method. Immunohistological
protocols were employed in evaluation of the liver’s pathological changes. The protein expressions of collagen type I (Col-I), extracellular signal-regulated kinase (ERK), TGF-beta receptor type I (TβR-I), TGF-beta receptor type II (TβR-II) and others against decapentaplegic homolog 7 (Smad 7) were assessed in the liver tissue by western blotting. Simultaneously, in order to systematically detect the axial and peripheral bone deterioration, rat lumbar and femoral bone densities were measured by dual-energy X-ray absorptiometry (DXA) in both groups. Besides, in the two groups, bone stereological analysis was performed by micro-computed tomography (micro-CT), and lumbar vertebral (L3) biomechanical properties were assessed.

Results: After modeling, compared with normal controls in group B, the serum levels of ALT and AST were remarkably increased in the rats in group A. The fibrotic/cirrhotic injuries in the liver tissue of rats in group A were also typically revealed by microscopy. The expression levels of Col-I, ERK and TβR in the liver tissue of rats were significantly higher in group A than in group B. Meanwhile, there was a significant difference of Smad 7 expression between the two groups (P<0.05). On the other hand, the lumbar and femoral bone mineral contents (BMC) and bone mineral densities (BMD) were significantly decreased in rats of group A compared with group B (P<0.01). Compared with the controls, moreover, bone histomorphometry and biomechanical test showed cancellous and cortical bone deterioration and biomechanical property markedly decline in LC rats (P<0.01).

Conclusion: A rat model of LC and LC-associated bone loss was established in this study. Our data furthermore suggest that LC can exert substantially destructive effects on qualitative, quantitative, microarchitectural and biomechanical properties of the bone.

PP 60
Study of Bone Metabolism: Bisphosphonate and Vitamin D in the Treatment of Ankylosing Spondylitis
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Objective: Ankylosing spondylitis (AS) is an inflammatory disease, which is characterized by involvement of the spine and joints. Its characteristic pathological changes are in tendons and ligaments and bony attachment point inflammation. This gradually progresses to joint stiffness and spinal deformity. Bone loss is common in AS, but the mechanism is not fully identified. Bisphosphonate, because of its role in bone metabolism and curative effect for osteoporosis (OP), has been increasingly used in the treatment of AS. This study was designed to detect the changes of bone alkaline phosphatase (BALP), tartrate-resistant acid phosphatase 5b (TRAP-5b), 25-(OH)D3 and Vitamin D receptor (VDR) before and after treatment, measure bone mineral density and record clinic indexes such as erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) level in AS. Another objective of this study was to investigate the related factors and the role of vitamin D in bone metabolism, discuss the treatment mechanism of bisphosphonate, and provide theoretical basis for the prevention and treatment of ankylosing spondylitis with osteoporosis.

Methods: Forty AS patients were studied, all of whom had the modified New York criteria for AS, without liver, kidney or any other bone diseases and never took glucocorticoids. Serum levels of BALP, TRAP-5b, 25-(OH)D3 and VDR were measured by avidin biotin peroxidase complex enzyme-linked immunosorbent assay (ABC-ELISA) and compared with that of normal control. Bone mineral density (BMD) was measured by dual-energy X-ray absorptiometry (DEXA). Clinical data collected included Ca, P, ESR and CRP. The correlations were analyzed between BMD and bone metabolism and the clinical parameters. The patients were divided into the Yunke treatment group and non-Yunke treatment group. We analyzed the differences between the treatments.

All the data were analyzed by SPSS17.0 for Windows statistical software. The mean number±s.d. was used to express the measurements. The t or t’ test was used to compare means. Chi-square test was used for the comparison of the categorical data. Linear correlation analysis was performed for correlations. P-value<0.05 was considered significant.

General description: The Yunke treatment group contained four female and eighteen male patients. The average age was (28±13) years, and the median duration was 5 years. The non-Yunke treatment group contained four female and fourteen female patients. The average age was (23±7) years, and the median duration was 2 years. There were no differences between the groups in age and gender (P>0.05). The normal control group contained four females and sixteen males. The average age was (30±10) years.

Results: (1) The prevalence of normal bone mass, osteopenia and OP in AS was 20%, 40% and 40%, respectively. The prevalence of osteopenia in lumbar, femoral neck, greater trochanter and inter-trochanter were 40%, 25%, 25% and 40%, respectively. The incidence of OP in lumbar, femoral neck, greater trochanter and inter-trochanter were 20%, 40%, 0% and 0%, respectively. There were different incidences of osteopenia and OP in different measurement sites (χ²=20.336, P=0.002) and the site with the highest incidence was the greater trochanter (65%).

(2) Serum levels of bone metabolic indexes: The serum levels of BALP and TRACP-5b in AS were significantly higher than normal controls (P<0.05). The serum level of 25-(OH)D3 was significantly lower than that of normal controls (P<0.05). The difference in the serum levels of VDR between the groups was not statistically significant (P=0.85). In both the Yunke group and non-Yunke group, the serum levels of BALP, 25-(OH)D3 and VDR increased significantly after treatment, and that of TRACP-5b reduced significantly (P<0.05). The absolute value of the differences before and after treatment was 0.770±0.266 µg l⁻¹, 2.073±1.633 ng l⁻¹, 3.269±1.146 µg l⁻¹ and 46.363±20.825 nmol l⁻¹, respectively, in the Yunke treatment group, and 0.207±0.152 µg l⁻¹, 6.659±1.716 ng l⁻¹, 1.011±0.643 µg l⁻¹ and 7.994±4.567 nmol l⁻¹, respectively, in the non-Yunke treatment group (P<0.05).

(3) In the Yunke treatment group, the serum level of Ca was significantly increased, and the levels of ESR, CRP, IgG, IgM, IgA, C3, C4, WBC and BASDAI were significantly decreased (P<0.05); in the non-Yunke treatment group, the serum level of P was significantly increased, and the levels of CRP, IgM,
C4, WBC, GLOB and BASDAI were significantly decreased. In both the two groups, the absolute values of ESR were 34.091±29.180 and 8.278±14.86 mm h⁻¹, respectively. The difference was statistically significant (P<0.05).

(4) The correlation between bone metabolic indexes and clinical indicators: According to linear correlation analysis, the level of 25-(OH)D₃ was positively correlated with P (r=0.809, P=0.000), the level of VDR was positively correlated with 25-(OH)D₃ and P (r=0.627, P=0.003; r=0.516, P=0.02, respectively); the level of BALP was negatively correlated with the levels of C₃ and GLOB (r=−0.526, P=0.017; r=−0.483, P=0.031, respectively); the level of TRACP-5b was negatively correlated with the levels of IgG, WBC, PLT and C₃ (r=−0.652, P=0.002; r=−0.550, P=0.012; r=−0.467, P=0.038; r=−0.554, P=0.011, respectively); the level of BALP was positively correlated with TRACP-5b (r=0.522, P=0.018).

(5) The correlation between BMD and bone metabolic and clinical indexes: According to linear correlation analysis, the BMDs of lumbar, femoral neck, greater trochanter and inter-trochanter were negatively correlated with BASDAI (r=−0.489, P=0.0029; r=−0.720, P=0.000; r=−0.686, P=0.001; r=−0.456, P=0.043, respectively). The BMD of femoral neck was negatively correlated with the course of disease (r=−0.636, P=0.003), and the BMD of intertrochanter was positively correlated with the level of BALP (r=0.472, P=0.036).

**Conclusions:** (1) AS patients had some degree of bone loss even in the early stage; most of them had osteopaenia or OP. For a better overall evaluation of BMD, it was necessary to measure BMD in multiple sites and then make a comprehensive analysis.

(2) There was abnormality in bone metabolism in patients with AS. High bone resorption was an important cause of bone loss, the degree of which was related to disease activity. It may be the pathogenesis of bone loss in AS.

(3) Lower level of vitamin D in AS may be one of the factors leading to or worsening osteoporosis.

(4) Abnormality in bone metabolism of AS was improved by therapy. Treatment with bisphosphonate was helpful to normalize bone metabolism and control the disease activity.

**PP 61**

**Correlation Between Synovial TRAF6 Expression and Bone Metabolism Markers in Rheumatoid Arthritis**

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**Background and Objective:** Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by polyarticular inflammation associated with synovitis, ostitis and periarticular osteopenia, often associated with erosion of subchondral bone and progressive joint space narrowing. Previous studies showed an abnormal activation of osteoclasts as well as altered skeletal bone metabolism and co-morbid conditions in RA. Tumor necrosis factor receptor-associated factor (TRAF) 6 is one of the critical modulators of the differentiation and resorption activity of osteoclasts. However, the pathophysiological role of TRAF6 in RA bone turnover is poorly understood. Here we evaluate the correlation between synovial TRAF6 expression and bone metabolism markers in RA.

**Methods:** Synovial tissue samples were obtained by needle biopsy from 22 patients with active RA. Semiquantitative analysis was performed to evaluate the intensity of TRAF6+ cells. Serum bone metabolism markers, including biochemical markers of bone formation (N-terminal propeptide of type I collagen, PINP and N-terminal midfragment of Osteocalcin, N-MID.OC), as well as markers of bone resorption (C-terminal telopeptide of type I collagen, CTX-I), were tested by chemiluminescence. Spearman’s correlation test was used to evaluate the correlation between TRAF6 expression and bone metabolism markers.

**Results:** Significant correlation was found between synovial TRAF6 expression and serum PINP level (r=0.427, P=0.047), as well as serum N-MID.OC level (r=0.517, P=0.014). No significant correlation was found between synovial TRAF6 expression and serum CTX-I level. Subanalysis of lining and sublining TRAF6 expression showed that only N-MID.OC correlated significantly with lining and sublining TRAF6 expression (r=0.507 and 0.464, P=0.016 and 0.029, respectively).

**Conclusion:** Synovial TRAF6 expression in RA correlated significantly with serum PINP and N-MID.OC level. Synovial TRAF6 was presumed to be involved in the pathogenesis of bone metabolism imbalance in RA.

**PP 62**

**Fluid Flow-Induced Calcium Response in Early or Late Differentiated Osteoclasts**

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**Objectives:** Intracellular calcium oscillation caused by receptor activator of nuclear factor kappa-B ligand has been demonstrated to promote the differentiation of osteoclasts. Osteoclasts are recruited on the surface of trabeculae, and are exposed to fluid flow caused by the deformation of the bone matrix. However, the roles of fluid shear stress (FSS) in calcium response and its signaling pathways during the differentiation process of osteoclasts are still unknown.

**Methods:** In the current study, the formation of tartrate-resistant acid phosphatase-positive, multinucleated osteoclasts from RAW264.7 macrophage cells were induced by co-culturing them with the conditioned medium from MC3T3-E1 osteoblasts. The in situ observations showed a high correlation between the area and the nuclear number of osteoclasts. The cells were stimulated by FSS at different levels (1 or 10 dyne/cm²) before (0 day) or after being induced for 4 or 8 days. The mechanically-induced calcium response was recorded and analyzed. The signaling pathways were also studied by using calcium-free medium, depleting the intracellular calcium stores, inhibiting the binding of ATP with its membrane receptor, blocking the mechanosensitive calcium channel (MSCC), blocking the voltage-sensitive calcium channel (VSCC), inhibiting the PLC pathway or blocking gap junction.

**Results:** The results indicated a different property of calcium oscillation for the osteoclasts in different fusion stages (i.e., more calcium-responsive peaks appeared in smaller osteoclasts than those in the larger ones). The rates of calcium influx decreased and the time of recovery in osteoclast cytosol...
increased along with the fusion of osteoclasts. In addition, increasing the FSS level enhanced the calcium oscillation of osteoclasts at early induction (4 days). However, this effect was weakened at the late induction (8 days). Almost all of the osteoclasts did not respond to shear flow when blocking MSCC, PLC or depleting the intracellular calcium stores, but part of the cells were able to respond on removing the extra-cellular calcium or blocking the ATP pathway.

Conclusions: The present work could help provide an understanding of the mechanism of the involvement of calcium in mechanically induced bone remodeling.

PP 63 Prevention of Wear Particle-Induced Osteolysis by a Novel V-ATPase Inhibitor, Saliphenylhalamide, through Inhibition of Osteoclast Bone Resorption

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Wear particle-induced peri-implant loosening (aseptic prosthetic loosening) is one of the most common causes of total joint arthroplasty. It is well established that extensive bone destruction (osteolysis) by osteoclasts is responsible for wear particle-induced peri-implant loosening. Thus, inhibition of osteoclastic bone resorption should prevent wear particle-induced osteolysis and may serve as a potential therapeutic avenue for prosthetic loosening. Here, we demonstrate for the first time that saliphenylhalamide, a new V-ATPase inhibitor, attenuates wear particle-induced osteolysis in a mouse calvarial model. In vitro biochemical and morphological assays revealed that the inhibition of osteolysis is partially attributed to a disruption in osteoclast acidification and polarization, both a prerequisite for osteoclast bone resorption. Interestingly, the V-ATPase inhibitor also impaired osteoclast differentiation via the inhibition of RANKL-induced NF-kB and ERK signaling pathways. In conclusion, we showed that saliphenylhalamide affected multiple physiological processes, including osteoclast differentiation, acidification and polarization, leading to inhibition of osteoclast bone resorption in vitro and wear particle-induced osteolysis in vivo. The results of the study provide proof that the new-generation V-ATPase inhibitors, such as saliphenylhalamide, are potential anti-resorptive agents for treatment of peri-implant osteolysis.

PP 64 Effects of High-Fat Diet on Bone Mineral Density and Microarchitecture in Low-Density Lipoprotein Receptor Gene Knockout (LDLR−/−) Mice and a Preliminary Study of its Mechanism

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Objective: To investigate the effect of high-fat diet on bone mineral density and microarchitecture in LDLR−/− mice and explore its mechanism.

Methods: There were 26 mice, including LDLR−/− mice (n=13) and sex-age-matched C57BL/6J mice (WT, n=13), involved in the study. By ordinary diet-adaptability feeding for a week, we executed three mice in each group. The rest of each group was randomly divided into ordinary diet and high-fat diet (ordinary diet+10% lard+2.5% cholesterol+0.5% bile acid sodium), including the WT ordinary diet group, WT high-fat diet group, LDLR−/− ordinary diet group and LDLR−/− high-fat diet group. All mice were killed at 40 weeks of age and left tibias were harvested to analyse the bone mineral density and bone microarchitecture by micro-CT. The parameters included tissue BMD (tBMD), structure model index (SMI), connectivity density (Conn.D), bone surface density (BV/TV), bone surface density (BS/BV), trabecular number (Tb.N), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp) and degree of anisotropy (DA). The right tibias were sampled for detection of OPG and RANKL mRNA expressions using real-time quantitative PCR, and by calculating the ratio of OPG/RANKL mRNA. A 2×2 factorial design was used in the processing with gene and diet as independent variable to investigate the effect of high-fat diet on bone calcium content, bone mineral density and microarchitecture.

Results: (1) At 12 weeks of age (baseline): compared with WT mice, there was a decrease of tBMD, BV/TV, Tb.N, Conn.D and OPG/RANKL mRNA ratio (P<0.05), and an increase of BS/BV, Tb.Sp, Tb.Th, RANKL mRNA level in LDLR−/− mice, but bone calcium content and DA had no significant change (P>0.05).

(2) At 40 weeks of age: (1) There was an interaction between LDLR−/− and high-fat diet in the changes of bone calcium content, tBMD, BV/TV (P<0.05), Tb.N, Tb.Th (P<0.01), SMI (P<0.01), Tb.Sp and the ratio of OPG/RANKL mRNA (P<0.05).

(2) High-fat diet had no significant change in bone calcium content, bone mineral density, microarchitecture and OPG/RANKL mRNA ratio (P>0.05). However, high-fat diet could decrease bone calcium content (P<0.01), tBMD (P<0.05), Tb.N, OPG/RANKL mRNA ratio (P<0.01) and increase Tb.Sp (P<0.05) in LDLR−/− mice. There was no change in Tb.Th, BV/TV and SMI (P>0.05). (3) Compared with WT mice, bone calcium content, tBMD and OPG/RANKL mRNA ratio (P<0.01) of LDLR−/− mice in ordinary diet were lower, the Tb.Sp was increased (P<0.05), but Tb.N Tb.Th, BV/TV, SMI had no change (P>0.05). In high-fat diet, LDLR−/− mice compared with WT mice showed a lower bone calcium content, tBMD, OPG/RANKL mRNA ratio (P<0.01), Tb.Th, BV/TV (P<0.05) and a higher SMI, Tb.Sp (P<0.05).

Conclusions: (1) LDLR−/− mice displayed a decreased bone mass and impaired microarchitecture. (2) At 40 weeks of age, both LDLR−/− and high-fat diet were associated with a decrease in bone mass and OPG/RANKL mRNA ratio and damaged microarchitecture. The most significant influences of bone mass, microstructure and OPG/RANKL mRNA were induced by interaction between LDLR−/− and high-fat diet. (3) The changes of bone mineral density and microarchitecture in LDLR−/− mice on high-fat diet might be caused by an increased bone resorption that was induced by the imbalance of the ratio of OPG/RANKL mRNA.
PP 65

Acid-Sensing Ion Channel 1a-Mediated Calcium Influx Regulates Survival of Osteoclasts
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It has long been known that systemic acidosis causes osteoclastic bone resorption. The increase in intracellular calcium ([Ca$^{2+}$]i) induced by extracellular acidosis contributes to regulation of the function of osteoclasts. However, the mechanisms by which acidification increases osteoclastic bone resorption remain largely unidentified. Interestingly, the Ca$^{2+}$-permeable acid-sensing ion channel 1a (ASIC1a) has also been demonstrated to act as an extracellular pH sensor in the central and peripheral nervous systems. The aim of the present study was to elucidate the apoptosis of ASIC1a in osteoclasts and to identify its function in regulating osteoclast survival through an acidosis-evoked increase in [Ca$^{2+}$]i. We found that ASIC1a was expressed during osteoclast differentiation from bone marrow-derived monocytes (BMMs) stimulated with the receptor activator of nuclear factor (NF)-κB ligand (RANKL) and macrophage colony-stimulating factor (M-CSF). In cultured osteoclasts, an extracellular pH of 6.0 increased acid-induced [Ca$^{2+}$]i. The ASIC1a-specific blocker PcTX venom and specific siRNA both significantly reduced this increase in acid-induced [Ca$^{2+}$]i, and inhibited acid-induced osteoclast survival. However, the increase in [Ca$^{2+}$]i was not observed in the absence of extracellular Ca$^{2+}$. Further, inhibition of ASIC1a-mediated Ca$^{2+}$ influx blocked acid-induced Nfatc1. These findings show that ASIC1a-mediated calcium entry plays a critical role in osteoclast survival by regulating activation of the transcription factor Nfatc1.

PP 66

A Research of Formation, Proliferation and Activity of Osteoblasts and Osteoclasts under Low-dose X-Irradiation in vitro
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Aims: Our previous researches showed that low-dose X-irradiation (LDI) can promote calcification mineralization, stimulate proliferation of osteoblast-precursors and osteoblastogenesis. In this study we focused on the role of LDI in formation and proliferation of osteoblasts and osteoclasts.

Methods: Osteoblasts and osteoclast precursors were separately and averagely randomized into the LDI group (cells exposed to an irradiation of 8 cGy) and SHAM group (cells exposed to sham irradiation). Proliferations of the two kinds of cells were evaluated by CCK8 assay; alkaline phosphatase (ALP) activity was measured by the p-nitrophenyl phosphate (PNPP) method, and mineralized nodule numbers were counted by the alizarin red S (ARS) staining method. The expression levels of BGP, BMP and ALP mRNA and protein in osteoblasts were evaluated by quantitative real-time polymerase chain reaction (Q-PCR) and western blotting. Osteoclast precursors were differentiated into osteoclasts in the presence of the receptor activator of nuclear factor-kB ligand (RANKL) and macrophage colony-stimulating factor (M-CSF). The morphology of these osteoclasts was examined using the tartrate-resistant acid phosphatase (TRAP) staining method. Q-PCR and western blotting were employed to quantify the expression of TRAP, Cathepsin-K, NFATc1 and MMP9 in osteoclasts. The apoptosis of osteoclasts was detected by the TUNEL reaction method.

Results: CCK8 assay showed that the proliferation of both osteoblasts and osteoclast precursors was accelerated significantly in vitro in the 8-cGy group compared to the SHAM group (P<0.05), while no significant changes of osteoclasts were observed between the LDI and SHAM groups by TRAP staining (P>0.05). The ALP activity and mineralized nodule in the LDI group were higher than those of the control groups (P<0.05). Q-PCR and western blotting analyses showed that the expression levels of BGP, BMP and ALP were significantly enhanced in the LDI group compared to the control group (P<0.05).

Conclusions: These observations suggested that LDI efficiently stimulated the formation, proliferation and activity of osteoclasts. Effects of LDI on the number and activity of osteoclasts were still unclear, though the proliferation of osteoclast precursors was also significantly enhanced. The apoptosis of osteoclasts was promoted by LDI.

PP 67

Effects of Neutral Buoyancy and Static Magnetic Field on Proliferation Properties in Osteocyte-like Cells MLO-Y4
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Aims: In the mission of deep space exploration, Taikonauts or biological objects would experience microgravity and an extremely weak magnetic field (hypomagnetic field). However, the role of hypomagnetic field and its influence on the function of biological organisms are still insufficiently understood. The purpose of this study is to investigate the effects of hypomagnetic field, moderate static magnetic field and neutral buoyancy-simulated weightlessness on bone remodelling.

Methods: Using neutral buoyancy-simulated weightlessness cell culture methods, osteocyte-like cells MLO-Y4 were exposed to a hypomagnetic field (magnetic field strength less than 0.5 μT, HMF), static magnetic field (200–400 mT, SMF) and geomagnetic field (50 μT, GMF) cell culture box, respectively. At 24, 48 and 72 h, cell proliferation, apoptosis, cycle and morphology were observed.

Results: Compared with the GMF group, the proliferation of MLO-Y4 cells was inhibited significantly in the HMF group at 48 and 72 h (P<0.01), and in the SMF group, the proliferation was promoted at the same time (P<0.01). In a different magnetic field environment, neutral buoyancy-simulated weightlessness could accelerate cell proliferation (P<0.01), which could offset the inhibition effect of HMF to a certain degree. Fluorescence microscope observation found that the cell apoptosis rate significantly increased at 72 h (25%) in the HMF group, and in the SMF group cell proliferation was luxuriant and cell apoptosis rate decreased at 48 h (10.2%) and 72 h.
A Novel Heterozygous Mutation in SLC34A3 Gene Causes Hereditary Hypophosphatemic Rickets with Hypercalciuria

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Aims: Hereditary hypophosphatemic rickets with hypercalciuria (HHHRH) is a rare metabolic disorder inherited in an autosomal recessive fashion, characterized by hypophosphatemia, short stature, rickets, and/or osteomalacia and secondary absorptive hypercalciuria. In two reports on six affected kindreds with HHHRH, the disease was mapped to chromosome 9q34, which contains the SLC34A3 gene that encodes the renal type 2c sodium-phosphate cotransporter. Our aim was to screen the SLC34A3 gene of one patient with typical manifestations of HHHRH to determine if there was a genetic contribution to the patient.

Methods: We described a 29-year-old man who had rickets as a child and had hypophosphatemia, bone pain, elevated 1,25(OH)2D level and recurrent nephrolithiasis. In two reports on six affected kindreds with HHHRH, the disease was mapped to chromosome 9q34, which contains the SLC34A3 gene that encodes the renal type 2c sodium-phosphate cotransporter. Our aim was to screen the SLC34A3 gene of one patient with typical manifestations of HHHRH to determine if there was a genetic contribution to the patient.


Conclusions: It has been reported that heterozygous SLC34A3 mutations lead to a variety of biochemical abnormalities. Our findings in this study suggest that the mutation in heterozygosity likely gave rise to a mild phenotype with different penetrance. The amino acid 468 of NPT2c is conserved among species (Arginine). The missense mutation likely disrupts the normal function of the transporter. Thus, this result raises some issues on the genetic basis and pathophysiological mechanism of hereditary hypophosphatemic rickets with hypercalciuria.

Poster abstracts
skeletal system. Beyond bone health, an emerging volume of scientific studies shows that vitamin D also provides other health (for example, cardiovascular, metabolic and immunological) benefits. In mainland China, there have been no systemic national surveys to depict the overall national vitamin D status. In order to provide the best possible evaluation of vitamin D deficiency/insufficiency in China, peer-reviewed English publications that measured plasma/serum 25-hydroxy-vitamin-D (25(OH-D) levels in various age groups of the Chinese since 2000 were collected and analyzed.

Results: A total of 18 investigations were found, which were conducted in 14 areas in the mainland with sample size ranging from several to a few thousands. Newborn, children and adolescence: In those not taking vitamin D-fortified products or supplementation, the 25-OH-D levels were far below adequate. In this group, as many as 40–90% had blood 25-OH-D levels lower than 50 nmol l\(^{-1}\). A study in Beijing showed even worse vitamin D status in school girls during the winter–spring period: over 40% had 25-OH-D levels under 12.5 nmol l\(^{-1}\). The very poor vitamin D status was not seen in subjects taking vitamin D-fortified products or supplementation, unequivocally suggesting that the supplementation generally raised vitamin D status. In addition, exposure to sunshine after the summer season improved the vitamin D status in subjects who were previously deficient.

Adults: Publications are limited, but as many as 70–90% of subjects not taking vitamin D-fortified products or supplementation had blood 25-OH-D levels under 50 nmol l\(^{-1}\), and 13–40% had levels below 25 nmol l\(^{-1}\).

Seniors: In this fast-growing population particularly vulnerable to bone fractures, the vitamin D status was also pessimistic. In two large-scale investigations in Beijing and Shanghai, as many as 70–90% of the subjects had blood 25-OH-D levels less than 50 nmol l\(^{-1}\). In a more recent study published in 2012, only 3.9% of 1460 subjects in the Shanghai urban area had blood 25(OH) D level above 75 nmol l\(^{-1}\).

Conclusion: From published investigations in China since 2000, vitamin D deficiency/insufficiency was found to be widespread and prevalent; this constitutes a significant but modifiable public health risk that deserves greater attention and more efficient and timely management.

PP 71
Analysis of the Therapeutic Effect of Proximal Humeral Fracture Treated with a Locking Proximal Humerus Plate in Elderly Patients
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Objective: The proximal humeral fracture is very common in old people due to osteoporosis. In this study, we investigated the therapeutic effects of senile proximal humeral fracture treated with an AO locking proximal humerus plate (LPHP).

Methods: From Dec 2007 to Dec 2010, 20 old patients with proximal humeral fractures were treated with LPHPs in our hospital; the average age was 60.2 years. The proximal humeral fractures were divided into different types according to the Neer classification; type II was 3 cases, type III was 9 cases and type IV was 8 cases. The surgeries were performed with the deltoid pectoralis major gap approach, and all the fractures were open reduced and fixed with LPHPs. Bone allograft was performed in six patients due to bone defects. The patients were regularly followed up after operation. Clinical follow-up data on each shoulder were obtained by physical examination and recorded according to CS shoulder score. Bone union, necrosis and the failure of internal fixation were evaluated by shoulder anteroposterior, lateral and axillary radiographic results.

Results: All patients in this study were followed up; the average duration of the follow-up was 18 months. According to CS shoulder score, 10 cases were excellent, 6 cases fair and 4 cases good. Three was no delayed union, malunion or disunion of fracture in this study.

Conclusion: Compared to the traditional plates, the LPHP has some advantages, such as flexible operation, reliable fixation, fewer complications and good functional recovery. The LPHP is especially suited to the comminuted fracture with osteoporosis in elderly patients, which can achieve satisfactory clinical results.

PP 72
The Relationship Between Vitamin D and Parathyroid Hormone: Calcium Homeostasis, Bone Turnover and Bone Mineral Density in Postmenopausal Women
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Introduction: Hypovitaminosis D can result in low bone mass, but not all patients with hypovitaminosis D develop secondary hyperparathyroidism. What this implies for bone metabolism and bone mineral density remains unclear. The aim of this study was to investigate the effects of hypovitaminosis D on bone turnover and the calcium metabolism and bone mineral density of patients with a blunted PTH response in comparison to patients with hypovitaminosis D and secondary hyperparathyroidism.

Method: 542 postmenopausal women (mean age: 64.0±10.8 years, mean YSM: 14.4±10.0) were evaluated by assessing serum calcium, phosphate, ALP, 25OHD, PTH, bone turnover markers such as BGP, CTX and P1NP, and BMD of lumbar spine, total hip and femur neck and so on. We defined hypovitaminosis D as 25OHD<10 ng ml\(^{-1}\) in the east China population according to our previous study; blunted PTH response was defined arbitrarily as a PTH within the standard laboratory reference range of 65 pg ml\(^{-1}\) in the presence of a 25OHD10 ng ml\(^{-1}\); hypovitaminosis D and secondary hyperparathyroidism was defined arbitrarily as a PTH above the standard laboratory reference range in the presence of a 25OHD10 ng ml\(^{-1}\); and vitamin D-replete subjects as 25OHD>10 ng ml\(^{-1}\).

Result: The prevalence of hypovitaminosis D in this cohort was 24%. Mean 25OHD varied across seasons (P=0.023), with women who had a visit during winter and spring having significantly lower 25OHD levels than those who had their visit during the fall (P=0.039 and 0.012). Serum PTH and bone turnover markers did not vary by season. All the subjects were divided into four groups by their serum 25OHD and PTH level. Group 1: 25OHD<10 ng ml\(^{-1}\) and PTH>65 pg ml\(^{-1}\) (7.0%); group 2: 25OHD>10 ng ml\(^{-1}\) and PTH<65 pg ml\(^{-1}\) (17.0%); group
3: 25OHD:10 ng ml\(^{-1}\) and \(\text{PTH}<65\ \text{pg ml}^{-1}\) (73.6%); group 4: 25OHD:10 ng ml\(^{-1}\) and \(\text{PTH}>65\ \text{pg ml}^{-1}\) (2.4%). Blunted PTH response was found in 70.8% of the patients with hypovitaminosis D. Patients with hypovitaminosis D and a blunted PTH response were characterized by a lower serum calcium level and a reduction in bone turnover (serum CTX and serum BGP), but no change in bone density as compared to those with hypovitaminosis D and secondary hyperparathyroidism. For spine BMD, the significant independent predictors were body mass index \((r^2=0.370; P<0.01)\), age \((r^2=-0.158; P<0.01)\), PTH \((r^2=-0.121; P<0.05)\) and serum CTX \((r^2=-0.118; P<0.05)\). For FN BMD, the significant independent predictors were years since menopause \((r^2=-0.201; P<0.01)\) and BMI \((r^2=0.139; P<0.05)\); for hip BMD, the significant independent predictors were age \((r^2=-0.239; P<0.01)\) and BMI \((r^2=0.239; P<0.01)\). There is little correlation between 25OHD and BMD.

**Conclusion:** This study identifies a high prevalence of hypovitaminosis D in healthy postmenopausal Chinese women, and a distinct group of patients with hypovitaminosis D and a blunted PTH response who show a disruption in calcium homeostasis but protected against PTH-mediated bone loss. Spine BMD is a more sensitive site with high serum PTH and high bone turnover.

**PP 74**

**Vitamin D Deficiency and Bone Mineral Density**

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**Objective(s):** To determine the frequency of vitamin D deficiency and insufficiency and its influence on bone mineral density (BMD) in patients of different regions of Ukraine.

**Materials and Methods:** We examined 1575 people aged 20–95 years living in different regions of Ukraine. 25-OH vitamin D and PTH level were evaluated by electrochemiluminescence method (Elecsys 2010, Roche). Vitamin D deficiency was diagnosed if 25-OH vitamin D was below 49.5 nmol l\(^{-1}\), vitamin D insufficiency between 74.5 and 50.0 nmol l\(^{-1}\), BMD was determined by ultrasound densitometry Sahara (Hologic) and DXA (Lunar).

**Results:** Vitamin D deficiency was registered in 81.8% of the persons; 13.6% examined had vitamin D insufficiency. There was a negative correlation between PTH and 25OHD \((r=-0.16, P<0.0000001)\). Secondary hyperparathyroidism was diagnosed in 11.9% patients. The mean level of 25OHD was significantly higher in southern residents of the country \((P<0.001)\) and during summer \((P<0.05)\). No significant correlation between 25OHD and BMD was found. Only patients with vitamin D deficiency had significant negative correlations between PTH level and BMD at the level of femur neck \((r=-0.12, P<0.004)\), dual femur \((r=-0.09, P<0.004)\), upper and lower extremities \((r=-0.11, P<0.01\) and \(r=-0.10, P<0.01\) accordingly) and forearm 33% \((r=-0.20, P<0.001)\).

**Conclusion(s):** In the Ukrainian population the frequency of vitamin D deficiency is 81.8%. Only patients with vitamin D deficiency have significant negative correlations between PTH level and BMD at the level of femur neck, dual femur, forearm 33%, upper and lower extremities.

**PP 73**

**The Effects and Safety of Early-Stage Drug Intervention in Chinese Postmenopausal Women at Risk of Osteoporosis on Bone Mineral Density and Bone Turnover by Alendronate 70 mg once bi-weekly**

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**Aim:** This study is designed to evaluate the efficacy and safety of early-stage drug intervention in Chinese postmenopausal women at risk of osteoporosis on bone mineral density and bone turnover by a new regimen of alendronate (ALN), 70 mg once bi-weekly, in Chinese postmenopausal women at risk of osteoporosis (osteopenia).

**Methods:** 180 Chinese women younger than age 70 with osteopenia were recruited, of which 90 participants received alfalcacidol 0.5 µg daily plus ALN 70 mg bi-weekly (ALN group) and the others received alfalcacidol 0.5 µg alone daily (control group). Bone mineral density (BMD) of the lumbar spine and total hip, serum C-telopeptide of type I collagen (CTX) and bone-specific alkaline phosphatase (BSALP) were measured during the 12-month treatment period.

**Results:** The results showed that, compared to the control group, the lumbar spine BMD and total hip BMD in the ALN group increased significantly. Serum BSALP levels decreased in both groups after treatment, but more dramatically in the ALN group than in the control group. Serum CTX level was suppressed after ALN treatment, but not in the control group. No serious adverse events were observed in either group. The safety profiles were similar between the two groups.

**Conclusion:** Based on this finding, it is concluded that early-stage drug intervention in Chinese postmenopausal women at risk of osteoporosis (osteopenia) with a new regimen of ALN, 70 mg once bi-weekly, is safe, well-tolerated and more effective than alfalcacidol alone in increasing BMD and reducing bone turnover rate.

**PP 75**

**Low Bone Mineral Density and Deficient Vitamin D in Native Chinese Rheumatoid Arthritis**

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**Aims:** Rheumatoid arthritis (RA) is a chronic disease characterized by inflammation of synovial joints, cartilage degradation and subsequent bone destruction. Factors including the disease activity itself, reduced daily physical activity, glucocorticoid use or menopause are likely to contribute to osteoporosis in RA. In this study we examined the risk factors for osteoporosis in RA. Bone mineral density (BMD) and its correlation with disease activity (modified DAS-28 score), serum 25-hydroxyvitamin D (25OHD) levels and disease duration were evaluated.

**Methods:** The study included 80 patients with rheumatoid arthritis fulfilling the American College of Rheumatology/ European League Against Rheumatism diagnostic criteria for
rheumatoid arthritis, and 112 age- and sex-matched healthy volunteers as controls at the same period (n=192). Erythrocyte sedimentation rate, C-reactive protein, rheumatoid factor and serum 25OHD (radioimmunoassay technique) were measured. Dual-energy X-ray absorptiometry (DEXA, QDR-4500, Hologic) was used to measure the BMD of the left femur at the time of recruitment. In the analysis of BMD, patients who had prosthesis in their joints were excluded. 

Results: Patients with RA had bone loss to various degrees: 35% had osteoporosis, 46% had osteopenia and 2.5% had normal BMD. BMD of the RA group were significantly lower than those of normal control (P<0.000). There were significantly lower levels of 25OHD in the RA group than in normal controls (P=0.001). In 38.8% of patients, serum 25OHD levels were between 4.0–10 ng/ml, 50% between 10–20 ng/ml, 10% between 20–30 ng/ml and 1.3% above 30 ng/ml. There was no difference in percentage of osteoporosis among RA females and males (P>0.05). The age in RA females was significantly correlated with their BMD (P<0.05, T=2.638). However, there was no correlation in the RA male group. By linear regression analysis, age, disease activity, serum 25OHD level and disease duration were significantly related to BMD in RA (F=10.417, P=0.000). Stepwise multiple regression analysis using BMD as the dependent variable resulted in age, DAS28, 25OHD and disease duration as independent variables. BMD correlated significantly (P<0.05) with the disease activity (r=−0.05, r=−0.261), serum 25OHD (P<0.05, r=0.338) and disease duration (r=−0.367).

Conclusion: Osteoporosis is more common in patients with RA than in the general population. The prevalence of concurrent osteoporosis is about 50%. In RA, the higher disease activity, the longer disease duration and the lower serum 25OHD level aggravate osteoporosis. This finding suggests that higher doses of vitamin D supplementation may be recommended not only for RA with reduced BMD but also for those with active disease.

PP 76
Risk Factors among Saudi Women for Low Bone Mass Density in Riyadh city: A Community-Based Study
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Objective: The objective of this study is to identify the important risk factors for low bone mass among Saudi women in Riyadh city.

Method: This is a community-based household cross-sectional survey. Fifteen hundred women were enrolled in Riyadh City starting from November 2010 till December 2011. Primary health care centers (PHCC) of the five regions of Riyadh city (north, east, west, south and center) were randomly selected, at the rate of one cluster from each region. Two-stage cluster sampling technique was used. Thirty clusters were selected proportional to the population size in each PHCC catchment area and from each cluster. Fifty households (HH) were randomly selected from each cluster to complete the required sample size. A structured questionnaire along with anthropometric measurements was administered. Quantitative ultra-sonography was done through the Achilles machine. Those whose readings were less than –1 and/or had undergone osteoporosis screening (OST) were referred to KKUH for DEXA measurements and blood investigations. These included Vit D level, bone profile, kidney and liver function tests, thyroid function test, and parathyroid hormone.

Results: Multivariate logistic regression analyses were done. Being illiterate (OR 2.97, 95% CI: 1.44–6.12, P<0.01) or of low level of education (OR 4.12; 95% CI 2.05–8.29, P<0.01), having a positive history of fractures on trivial fall (OR 2.20; 95% CI 1.03–4.69, P=0.04) and not drinking laban (OR 2.81; 95% CI 1.47–5.37, P<0.01) were all significantly associated with low BMD. Increase in weight in kg was found to have a protective effect on the low BMD (OR 0.98; 95% CI 0.96–0.99, P=0.04).

Conclusion: Educational level, history of fracture, drinking laban and weight were found to be important risk factors.

PP 77
A Prospective Multicenter Study of Medication Patterns and Determinants of Outpatients with Reduced Bone Mineral Density and Increased Fragility Fracture Risks
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Objectives: To investigate medication patterns and determinants in outpatients with reduced bone mineral density (BMD) and increased fragility fracture risks in a prospective multicenter study.

Methods: A prospective, non-interventional study was conducted of outpatients who had a cervical or lumbar (L1–L4) T score ≤−1 by bone densitometry and who had increased fracture risk and at least one risk factor for fracture. During 1 year follow-up, BMD, CTx/Cr and rate of fragility fracture were recorded at the 3rd, 6th and 12th months, respectively. Patient health status was determined using the EQ-5D questionnaire.

Results: 1803 patients, including 382 males and 1421 females were eligible, with a BMD of 22.93±3.21 g cm−2 (range, 13.71–38.27) and a cervical and lumbar T score of −1.91±0.37 (range, −6.00 to −5.90) and −2.14±1.31 (range, −6.20 to −3.30), respectively. At baseline, 22.96% of the patients took bisphosphonates, active vitamin D and calcium supplement (vs 31.77% at 12 months), 16.2% took bisphosphonates and active vitamin D (vs 17.72% at 12 months), 13.59% took active vitamin D and calcium supplement (vs 14.63% at 12 months), and 7.21% took bisphosphonates and calcium supplement (vs 8.43% at 12 months). The cervical and lumbar T score was −1.82±0.93 (range, −4.90 to −2.50) and −1.85±1.20 (range, −5.40 to −3.30), respectively, at 12 months (P<0.0001 vs baseline). The CTx/Cr ratio was 179.35 and 133.39 mg m−2.5 (range, 5.40 to 3.30), respectively, at 3 and 6 months (P<0.0001 in both vs baseline, 234.15 mg m−2.5). The EQ-5D score was 81.29 at 12 months (P<0.0001 vs baseline, 76.41). The percentage of patients with falls within the previous 12 months decreased (baseline, 11.16% vs 12 months, 6.82%). The degree of physical activity, prior diagnosis of decreased BMD and course of osteoporosis, age >50 years and current use or use of corticosteroids for over 3 months were significant factors for patients being prescribed with bisphosphonates. The degree of physical activity, history of fragility
fracture, prior diagnosis of decreased BMD and osteoporosis, and current use or use of corticosteroids for over 3 months were significant determinants of patients being prescribed active vitamin D. Prior diagnosis of decreased BMD was a significant determinant of patients being prescribed bisphosphonates and active vitamin D. Body mass index, family history of fragility fracture, current use or use of corticosteroids for more than 3 months and diagnosis of rheumatic arthritis were significant determinants of patients being prescribed calcium supplement.

Conclusion: In real-life practices, medical therapy improves the outcome of outpatients with BMD and increased fragility fracture risks. Analysis of medication patterns and determinants could help in rational use of medication for at-risk patients.

PP 78 Detection of Polymorphism in Hydroxyapatite and Bone of Osteoporotic Patients Treated with Bisphosphonates

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Objectives: The aim of the present study was to evaluate physicochemical characteristics of the processes taken place in the surface of bones in patients with osteoporosis, treated with alendronate (A) and risedronate (R). Currently, (P-C-P) bisphosphonate is used as a drug because it is an antiresorptive agent and its effect on fibrous tissue is also being studied. There are more than twenty biologically active compounds of the family of bisphosphonates, which act specifically in bone diseases such as osteoporosis, imperfect osteogenesis, Paget or different kinds of cancer.

Methods: (A) and (R) were used to treat bone samples and synthetic hydroxyapatite (Ha). The effects of (P-C-Ps), as an antiresorptive at the bone level, in bones and in synthetic hydroxyapatite (Ha), were studied in vitro assays. First, nano hydroxyapatite was synthesized by the sol/gel method. Samples obtained were analyzed by X-ray diffraction and by Scherrer’s equation to determine crystal size. It was compared with healthy human bone, obtaining an excellent correlation. The lattice parameters were determined by the Rietveld method. SEM was used to obtain the Ca/P ratio and TEM to study the microstructure. Second, in vitro treatment simulating the natural conditions in which the drug interact with the patient’s bones and synthetic hydroxyapatite was performed using (A) and (R). After 30 days, the samples were characterized with a laboratory diffractometer (Bragg-Brentano geometry) at room temperature with a HTK camera, Philips Nereleco Diffractometer PW 3710. Samples were also analyzed in the XRD beamline of the LNLS Sincrotr;├ö├ç├┐on. FTIR analysis were carried out with a Nicolet 520 P, by RD. Differential scanning calorimetry (DSC) was carried out with a TA Instrument Q20. The Ca/P ratio was calculated by EDX using FIT, SEM, Carl Zeiss, NTS and Mod. Supra 40.

Results: The treatments used produced noteworthy changes in the surface of the materials. None of the analysis methods used was able to match a known polymorph to that found on the surface of the bone. This ascertained that its composition was the same, in all cases, to that of the known polymorphs. On the other hand, the samples treated with (A) showed, after a certain time, trace of the same structure as those treated with (R).

Conclusions: New polymorphs were formed in the treated surfaces; besides, they have been characterized, but not identified as any of those stated in the literature.

PP 79 Menatetrenone vs Alfalcaldol in the Treatment of Postmenopausal Women with Osteoporosis in China

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Aims: Postmenopausal osteoporosis is a serious public health problem in the world. Low vitamin K consumption is associated with a higher risk of hip fracture among older women and men, and lower bone mass in older women and men. The aim of the study is to evaluate the efficacy and safety of menatetrenone (vitamin K2) treatment in postmenopausal women with osteoporosis in China.

Methods: The study recruited healthy postmenopausal osteoporotic women aged between 45 and 75 years. T-score of lumbar spine (L2–L4) and/or femoral neck BMD was lower than −2.0. The patients were randomized to receive either menatetrenone (Eisai) 15 mg, three times per day, or alfalcaldol (Haier) 0.25 μg, twice per day for one year. Patients also received elemental calcium 500 mg. BMD and biochemical markers including serum osteocalcin (OC) and undercarboxylated osteocalcin (ucOC) were measured at M0, M6 and M12.

Results: After 12 months of treatment, BMD was significantly increased by 1.2% and 2.7% at the lumbar spine and trochanter, respectively, in the menatetrenone group (M group) compared with baseline BMD (P<0.001). In the alfalcaldol group (A group), BMD was also increased by 2.2% and 1.8% at the lumbar spine and trochanter, respectively (P<0.001). There were no changes of BMD in the femoral neck in both groups. No differences were found between the two groups after 6 and 12 months of treatment (P<0.001). After 12 months treatment, OC and ucOC were decreased by 38.7% and 82.3% in the M group compared with baseline (P<0.001). In A group, OC and ucOC were also decreased by 25.8% and 34.8%, respectively (P<0.001). The decline of serum OC and ucOC was greater in M group than in A group (P<0.001). The ratio of ucOC/OC also decreased after treatment, especially in M group (M group P<0.001, A group P<0.05). The safety profile of menatetrenone was similar to that of alfalcaldol.
Conclusions: With one-year treatment by menatetrenone, the BMD of lumbar spine and hip increased in postmenopausal osteoporotic patients. Menatetrenone treatment, compared with alfacalcidol, has similar effects on BMD improvement. The biochemical markers of bone metabolism (OC, ucOC and ucOC/OC) decreased significantly after treatment in both groups. However, menatetrenone is more powerful in reducing these three parameters. It is a good choice in the treatment of postmenopausal osteoporosis in China.

PP 80
Clinical Observation of Bushen Zhuanggu Granules in Improving the Bone Metabolism of Aged Males with Osteoporosis
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Objective: To observe the clinical efficacy of Bushen Zhuanggu Granules in increasing bone mass and improving bone metabolism in aged males through comparison within a Bushen Zhuanggu Granules group before and after treatment, and comparison between the Bushen Zhuanggu Granules group and an Alendronate Sodium Tablets or Caltrate D group after treatments.

Methods: The levels of serum PINP, β-Crossslaps and N-MID were tested by electrochemiluminescence immunoassay; the BMD of lumbar vertebrae 1–4, femoral neck, Ward’s triangle, trochanter and femur were measured with an American GE company’s Lunar Prodigy dual-energy X-ray (DEXA) bone densitometer; the subjects were divided into three groups: the Bushen Zhuanggu Granules group was given Bushen Zhuanggu Granules+Caltrate D, the Alendronate Sodium Tablets group was given Alendronate Sodium Tablets+Caltrate D, and the Caltrate group D only was given Caltrate D.

Results: After 6 months’ treatment, PINP, β-Crossslaps and N-MID of the Bushen Zhuanggu Granules Group decreased significantly, and BMD of the left femoral neck, Ward’s triangle and left femur increased significantly. Compared with the Caltrate D group after treatment, the Bushen Zhuanggu Granules group showed better efficacy in improving bone metabolism and increasing BMD in elderly men, while compared with the Alendronate Sodium Tablets group, the Bushen Zhuanggu Granules group showed a similar efficacy.

Conclusion: Bushen Zhuanggu Granules are a safe and effective medicine for the treatment of senile osteoporosis; it increases bone mass and improves bone metabolism in aged males with osteoporosis.

PP 81
Icariin Increases Bone Mass and Bone Strength in Osteoprotegerin-deficient Mice through the Activation of BMP Signaling
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Introduction: Osteoporosis is defined as reduced bone mineral density with a high risk of fragile fracture. Current available treatment regimens include anti-resorptive drugs such as estrogen receptor analogs and bisphosphates and anabolic agents such as parathyroid hormone (PTH). However, neither option is completely satisfactory because of adverse effects. It is thus highly desirable to identify novel anabolic agents to improve future osteoporosis treatment. Icariin, the main active flavonoid glucoside isolated from *Epimedium pubescens*, has been reported to enhance bone healing and treat osteoporosis, but its effect on bone formation in vivo and the underlying mechanism remain unclear.

Methods: In this study, to explore the efficacy of alternative bone anabolic drugs in the treatment of osteoporosis, we investigated the in vivo effects of Icariin on bone formation using local calvarial injection and the Osteoprotegerin (OPG)-deficient mouse model.

Results: Calcein double labeling of undecalcified skull tissue sections showed that Icariin stimulates new bone formation after local injection over the surface of calvaria. Histomorphometric analysis revealed significant and dose-dependent increases in bone formation rate (BFR) and mineral appositional rate (MAR) in Icariin-treated groups, compared to the control group. We found that Icariin is also capable of significantly reversing OPG-deficient-induced bone loss and promotes osteoblast differentiation in OPG-deficient mice, which is demonstrated by the upregulation of the RNA expression of BMP2 (2.2-fold), BMP4 (2.1-fold), RUNX2 (1.9-fold) and OCN (3-fold). We also examined the effect of Icariin on the biomechanical properties of the femoral diaphysis and found that it significantly enhances bone mechanical strength, increasing the maximal force, bone stiffness and bone energy.

Conclusion: Taken together, we found that Icariin increases bone mass and bone strength in OPG-deficient mice through the activation of BMP signaling. All the results demonstrate that Icariin could be a potential anabolic agent to stimulate bone formation and prevent bone loss.

PP 82
Relations of Circulating Matrix Metalloproteinase-3 and Osteopontin Levels with Bone Mineral Density and Biomarkers in Postmenopausal Women
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Background: Osteoporosis is a metabolic condition characterized by decreased bone mass and strength due to increased bone turnover, which compromises bone architecture and results in increased fracture risk. Postmenopausal osteoporosis is the most common type of osteoporosis associated with estrogen deficiency. However, the mechanisms by which estrogen deficiency causes bone loss are complex and are not fully understood. Matrix metalloproteinase (MMP) forms a family of zinc-dependent proteases essential for several physiological and pathological events, such as embryonic development, angiogenesis, wound repair, periodontal disease, rheumatoid arthritis, cancer invasion and metastasis. Osteoblast-derived MMPs have been shown to play a role in bone metabolism by degradation...
of the bone matrix. Bone resorption is dependent on the activity of MMP-3, which degrades denatured type I collagen and other components of the bone organic matrix. Evidence has accumulated for an active participation by osteoblast-derived MMPs in the initiation of bone resorption and coupled bone formation by degrading the unmineralized osteoid layer of the bone surface to allow osteoclasts to attach to the mineralized matrix. In vitro, normal osteoblasts secrete abundant MMP-3, which is triggered by osteopontin (OPN); then the OPN–MMP-3 complex is formed to induce bone matrix into ossification before bone mineralization.

**Objective:** To study matrix metalloproteinase-3 (MMP-3) and osteopontin (OPN) levels, and correlations of MMP-3 and OPN with bone metabolic markers and bone mineral density (BMD) in postmenopausal Chinese women.

**Methods:** MMP-3, OPN, osteoprotegerin (OPG) and osteoprotegerin ligand (OPGL) of 120 postmenopausal Chinese female volunteers were measured using ELISA. BMD was measured using dual-energy X-ray absorptiometry. Women were classified as normal, osteopenic and osteoporotic, according to the WHO criteria.

**Results:** (1) OPN concentrations were significantly higher in osteoporotic women (56±20 ng ml⁻¹) than in normal women (26±11 ng ml⁻¹) (P<0.05), but MMP-3 concentrations were little higher in osteoporotic women (153±121 ng ml⁻¹) than in normal women (125±101 ng ml⁻¹) (P<0.05). (2) In osteoporosis, notable negative correlations between OPN, ratio of OPN/MMP-3 and BMD, and sOPGL were found (P<0.05), as well as positive relations between OPN, ratio of OPN/MMP-3 and sOPG (P<0.05), and positive relation between the MMP-3 and BMD of Wards triangle (P<0.05); in osteopenia, negative relations between MMP-3 and BMD, as well as ratio of OPN/MMP-3 were detected (P<0.05).

**Conclusion:** There are significant correlations between serum OPN, and ratios of OPN and MMP-3, and bone biomarkers of sOPG, sOPGL, OPN and OPN/MMP-3 may increase with high bone metabolism. The increases in OPN and ratio of OPN/MMP-3 appear possibly as a concomitant event in the high bone-turnover state, such as postmenopausal osteoporosis.

**PP 84**

**Osteoporosis Awareness and Related Health Behaviors in Postmenopausal Chinese Females**

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**Aims:** The purpose of this study was to assess osteoporosis beliefs and osteoporosis preventative behaviors in community postmenopausal women, and to explore the associations between osteoporosis beliefs and bone mineral density (BMD).

**Methods:** Using a multi-stage sampling method, 308 Chinese Han postmenopausal women were selected from non-aca-
Posters abstracts

PP 85
The Effect of Long-Term and High-Dose Esomeprazole Use on Bone Metabolism and Bone Mineral Density in Adult Rats
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Background: Proton pump inhibitors (PPIs) are widely utilized for the treatment of acid-related disorders by blocking the gastric acid pump, H+/K+-adenosine triphosphatase (ATPase). But there have been a number of studies demonstrating an association between PPI use and an increased risk of hip fracture among the people who have had long-term use of PPIs in the recent years. In the present study, we examined the effect of long-term and high-dose esomeprazole use in rats.

Methods: Twenty-four rats (3 months old) were divided into three groups randomly by weight: control group (received vehicle), routine-dose esomeprazole group (treated with esomeprazole 10mg per kg per-day) and high-dose esomeprazole group (treated with esomeprazole 50mg per kg per-day). Dual-energy X-ray absorptiometry (DXA), enzyme-linked immunosorbent assay (ELISA) and an automatic chemistry analyzer were used to assess total bone mineral density (BMD), bone alkaline phosphatase (B-ALP), and serum calcium and phosphorus concentrations at the beginning and after 8 and 15 weeks, respectively. Bone histomorphometric analysis was also performed to evaluate the structural changes in rats’ femoral head section.

Results: The respondents’ mean age was 54.5±6.15 years, and retired women comprised approximately 45% (44.8%) of the 308 samples. The total osteoporosis health belief score was 66.12±5.55 in postmenopausal women. 78 (25.3%) reported osteoporotic fracture history, 77 (25.0%) of the participants never took milk or dairy products. 117 (38.0%) of the participants took bean foods occasionally (less than 500 g per week). 16 (5.2%) were smokers, and 67 (21.7%) of the subjects drank alcohol 1–2 times per week. Among 161 (52.3%) maintained regular exercise (30min and at least 3 times per week). Among the 100 participants randomly selected, 32 women had osteoporosis, and 54 had osteopenia. 23 women had the osteoporotic fracture history. The logistic regression analysis by health beliefs to BMD results showed that although all the seven aspects entered into the regression equation, only perceived benefits of exercise was significantly correlated with BMD at the femoral neck.

Conclusions: Postmenopausal women express concern about osteoporosis, but exhibit relatively low health beliefs and inadequate health behaviors. The findings from the study suggest the need for large community-based studies so that high-risk populations can be picked up and early interventions and other life-style changes can be implemented.

PP 86
Are Bone Mineral Density Loci also Associated with Hip Osteoporotic Fractures? A Validation Study on the Previously Reported Genome-Wide Association Loci in a Chinese Population
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Objective: Osteoporosis is a highly heritable disease characterized mainly by low bone mineral density (BMD) and osteoporotic fractures (OF). Most genome-wide association studies (GWAS) on osteoporosis have focused on BMD, whereas little effort has been expended to identify genetic variants directly linked to OF.

Methods: To determine whether BMD loci are also associated with OF risk, we performed a validation study to examine 23 BMD loci reported by recent GWAS as having an association with hip OF risk. Our sample consisted of 700 elderly Chinese Han subjects, 350 with hip OF and 350 healthy matched controls.

Results: Through this study we identified four BMD loci that were also associated with hip OF in the Chinese population, including 7q21 (FLJ42280, P=1.17×10^{-4} for rs4729260; P=0.008 for rs7781370), 6p21 (MHC, P=0.004 for rs3130340), 13q14 (TNFSF11, P=0.012 for rs9533090; P=0.018 for rs9594759; P=0.020 for rs9594738; and P=0.044 for rs9594751) and 18q21 (TNFRSF11A, P=0.015 for rs884205). In particular, SNP rs4729260 at 7q21 remained significant even after conservative Bonferroni correction.

Conclusion: Our results further highlight the importance of these loci in the pathogenesis of osteoporosis, and demonstrate that it is feasible and necessary to use OF as the direct phenotype to conduct genetic studies, which will help enhance our understanding of the genetic architecture of osteoporosis.
**PP 87**

Teriparatide [rhPTH (1-34)], but not Strontium Ranelate, Demonstrates Bone Anabolic Properties in Osteopenic Ovariactomized Rats

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We compared the effect of strontium ranelate (SR) and teriparatide (TPTD) on gene expression, BMD, biomechanical properties and histomorphometry in a rat model of estrogen-deficiency osteoporosis. Eight-months-old rats were ovarioctomized (Ovx) at age 6 months and permitted to lose bone for 2 months before treatment for 3 or 12 weeks with TPTD (5 or 15µg per kg per day s.c.) or SR (150 or 450mg per kg per day p.o.). After 3 weeks of treatment, RT–PCR analyses of the distal femur showed TPTD elevation of collagen 1a2 (Col 1a2), osteocalcin (OCN), alkaline phosphatase (ALP), bone sialoprotein (BSP) and Runx2 gene expression at both doses, relative to Ovx controls. SR had no effect on these genes at either dose. Neither compound affected osteoprotegerin expression. Compared to Ovx controls, 12 weeks of TPTD5 and TPTD15 treatment increased lumbar vertebral (LV) BMC and BMD, while SR150 and SR450 increased only BMC. Further, TPTD5 and TPTD15 increased LV strength and stiffness (56%, 68%), while neither SR150 nor SR450 had significant effects on vertebral strength, and only SR450 had a 27% effect on stiffness. There were dose-dependent effects of TPTD5 and 15 on strength (36, 57%) at the proximal femur, but not for SR at either dose. At the femoral midshaft, there were dose-dependent effects of TPTD5 and 15 on BMD and BMC, and TPTD15 increased strength (22%). SR150 increased midshaft BMC; SR450 increased BMD and BMC, but neither dose of SR significantly affected strength. Histomorphometry at the proximal tibial metaphysis (PTM) showed dose-dependent effects of TPTD5 and 15 on trabecular thickness and number, and relative bone volume and formation rates. There were no significant effects of SR on histomorphometric parameters. SR BMD and BMC efficacy could be explained by incorporation of SR into bone, but neither dose stimulated an anabolic bone response and did not significantly improve the bone biomechanical properties of Ovx rats. These findings confirmed the bone anabolic efficacy of teriparatide, but not strontium ranelate in mature osteopenic, Ovx rats.

**PP 88**

Chinese Herbal Medicine for Osteoporosis: A Systematic Review

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To evaluate the effectiveness of Chinese Medicine in the treatment of osteoporosis. A literature search was performed using the phrase ‘Chinese herb AND herbal AND osteoporosis AND fracture’ with the limits ‘randomized controlled trial’ using databases including MEDLINE (1966 to Feb. 2012), EMBase (1974 to Feb. 2012), Chinese Biomedical Literature Database (CBM, 1978 to Feb. 2012), CJFD (CNKI, 1994 to Feb. 2012), the Chinese Scientific and Technical Journals database (VIP, 1989 to Feb. 2012), wanfang data (1995 to Feb. 2012), database of Chinese biomedical journals (CMCC, 1994 to Feb. 2012), China Doctoral Dissertations Full-text Database (CQFD, 1984 to Feb. 2012), China Master’s Theses Full-text Database (CMFD, 1984 to Feb. 2012) and aCPFD (2000 to Feb. 2012). At the same time, we searched the references of the included studies. Information was carefully extracted from all eligible publications independently by two of the authors of the present study. We also graded the methodology quality of all included trials by JADAD and ‘Risk of Bias table’, which was recommended by Cochrane handbook 5.0, and extracted the data independently. Differences in the extraction of data were solved by a discussion. A meta-analysis was performed using RevMan 5 if there was no significant heterogeneity. We described the dates that could not be combined. This meta-analysis followed the PRISMA statement guidelines. According to measurement indicators, interventions and outcomes (which included BMD of the osteoporosis patients, clinical symptoms and the risk of fracture), a subgroup analysis was performed. The study aims to evaluate the effects of the Chinese herb on the treatment of osteoporosis, the methodology quality of the clinical reports and the chosen outcome. The pooled data showed that the percent change of increase in BMD in the spine is higher with Chinese herb compared to placebo (lumbar spine: WMD=0.06, 95% CI: 0.06–0.09, P<0.01). In the femoral, Chinese herb showed significantly higher increments of BMD compared to placebo (femoral neck: WMD=0.05, 95% CI: 0.04–0.07, P<0.01). Compared to the other standard anti-osteoporotic drugs, Chinese herbs also show an advantage in BMD change (lumbar spine: WMD=0.02, 95% CI: 0.01–0.04, P=0.002; femoral: WMD=0.01, 95% CI: –0.01 to –0.02, P=0.37). Our results demonstrated that Chinese herb significantly increased both lumbar spine BMD and femoral BMC compared to the placebo or other standard anti-osteoporotic drugs.

**PP 89**

Association of GC and CYP2R1 Genetic Variants with Serum Vitamin D Concentrations in Postmenopausal Women of the Han Ethnic Group in Beijing

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Objective: Vitamin D deficiency is a common and important health problem. Recently, some genetic factors were found to influence serum 25(OH)D concentration. This study is to determine if GC and CYP2R1 genetic variants are associated with serum 25(OH)D level, bone mineral density (BMD), bone turnover markers and osteoporotic fracture in postmenopausal women.

Methods: We randomly selected a population of 1494 postmenopausal women of the Han ethnic group from 7 communities in Beijing. The main information acquired was as follows:
osteoporotic fracture and vertebral fracture phenotypes: questionnaire and vertebral X-ray reading; BMD at lumbar spine (L2–4), femoral neck (FN) and total hip by dual-energy X-ray absorptiometry (DXA); serum bone turnover markers and 25(OH)D levels by automated Roche electrochemiluminescence system; genotyping by TaqMan pre-designed SNP genotyping assays in a real-time PCR system. Linear regression and binary logistic regression were used to test the associations between SNP genotypes and haplotypes with serum 25(OH)D level, BMD, bone turnover markers, and the risk of low bone mass, osteoporotic fracture or vertebral fracture.

**Results:** (1) 1338 women have vitamin D deficiency and another 146 women have vitamin D insufficiency, accounting for 89.6% and 9.8% of the total sample, respectively. (2) The variants of rs222020 ($P=0.003$) and rs2298849 ($P=3.73e^{-4}$) at GC are significantly associated with serum 25(OH)D level. This association is still significant after 10000 times max(T) permutation test. Allele C of rs222020 and allele G of rs2298849 might be protective for serum 25(OH)D level. Among the haplotypes of rs222020–rs2298849, CG ($β=0.138$, $P=0.001$) and TA ($β=-0.118$, $P=0.002$) correspond to increasing and decreasing serum 25(OH)D concentrations, respectively. We did not find any significant association between CYP2R1 polymorphisms and serum 25(OH)D level. (3) No significant association was found between GC polymorphisms and BMD at L2–4, FN and total hip, β-CTX, P1NP, and the risk of low bone mass, osteoporotic fracture or vertebral fracture. CYP2R1 polymorphisms had similar results except for its significant association with β-CTX and P1NP.

**Conclusion:** GC variants have significant association with serum 25(OH)D level among postmenopausal women of the Han ethnic group in Beijing. CYP2R1 variants had no significant association with serum 25(OH)D level, but had association with β-CTX and P1NP level.

**PP 90**

**Analysis of the Difference between Osteoporosis Fracture and Non-Traumatic Femoral Head Osteonecrosis of Bone Quality and Biochemical Markers**

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**Objective:** To evaluate the potential differences in bone quality and biochemical marks between the patients with osteoporotic fracture and non-traumatic femoral head osteonecrosis.

**Methods:** With the permission of the Ethics Committee of the Hospital and the patients, the femoral heads of patients were collected 2 h after the surgery for total hip replacement. 52 subjects with fragility fractures (mean age 72.2±9.6 years) and 77 subjects with non-traumatic femoral head osteonecrosis (mean age 56.6±12.4 years) were evaluated. The cortical bone was removed from surgical samples, and then cancellous bone specimens (6 mm×6 mm×7 mm) were obtained from femoral heads along the plane perpendicular to the stress direction of bone under physiological conditions. Morphologic and mechanical analysis was performed on surgical samples, such as DXA scan, three-dimensional microstructure scan with micro-CT, mechanical experiments, ash weight, demineralization and microdamage parameters measurement. Meanwhile, serum and urinary bone markers were assayed in 59 patients with osteoporosis fracture and 105 patients with non-traumatic femoral head necrosis.

**Results:** (1) Bone mass: compared with the non-traumatic osteonecrosis group, the osteoporosis group was statistically less significant in volumetric bone mineral density, tissue bone mineral density and bone mineral content measured by DXA. (2) Microstructure: compared with the other group, the osteoporosis group was statistically less significant in bone mineral content, area, bone mineral density, bone trabecular number and bone volume fraction ($P<0.05$), while the osteoporosis group was statistically more significant in the trabecular separation and structure model index and degree of anisotropy ($P<0.05$), but the difference between them was not statistically significant in trabecular bone thickness and bone area density. (3) Inorganic and organic qualitative content: osteoporosis group was statistically less significant in volumetric ash content and percentage of ash content ($P<0.05$), while it was more significant in percentage of organic content ($P<0.05$); but it was not statistically significant in volumetric organic content. (4) Bio-mechanics: the osteoporosis group was statistically less significant in elastic stress, elastic modulus and maximum stress ($P<0.05$), while it was highly significant in change of height after fatigue test ($P<0.05$). (5) Bone metabolic marker: the osteoporosis group was statistically less significant in serum TRACP-5b index content ($P<0.05$), while the differences were not statistically significant in serum BAP, BGP, urine CTX and urine creatinine indexes. (6) Parameters of microdamage: the difference in mean microcrack length, microcrack density and microcrack surface density were not statistically significant.

**Conclusion:** Our data show significant differences in bone quality and biochemical marks in the two groups. Compared with the osteonecrosis group, the osteoporosis group maintains the ability of fracture resistance mainly by enhancing the anisotropy of trabeculae, while the osteonecrosis group achieves primary bone strength by increasing the trabecular separation, number of trabeculae and structure model index. The arrangement of organic collagen fiber is different in the two diseases, and the peak osteoblast activity of the osteoporosis group is more obvious than the other.

**PP 91**

**Generation and Characterization of Mice with Conditional Glucocorticoid Receptor Knockout in Cartilage**

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**Objective:** The mechanisms by which glucocorticoids (GCs) exert their receptor-mediated effects on cartilage and bone cells are poorly understood. We aimed to elucidate the role of the glucocorticoid receptor (GR) in chondrocytes, and its
interaction with bone, through characterisation of a cartilage-specific GRKO mouse line.

**Methods:** GR\textsubscript{fox/flox} mice were crossed with a transgenic mouse model (Col2a1-CreER\textsuperscript{T2}) to generate tamoxifen-inducible Col2-GRKO mice, in which the cre recombinase is expressed under the control of type II collagen (Col2) promoter. GRKO was induced at 2, 4 and 10 weeks of age by daily i.p. injection of tamoxifen (1 mg per 10 g BW per day) for 5 days. Mice were then monitored for 8 weeks for body weight and long bone growth. Bones were analysed by histology and micro-CT at end point.

**Results:** Body weight and bone length were similar in GRKO and WT mice in all the three age groups. Histology of knee joints, growth plates and intervertebral discs showed normal cartilage structure in both GRKO and WT mice. However, compared with WT littermates, male GRKO mice induced at 10 weeks of age displayed significantly increased BV/TV (19.6\% vs 13.9\%) and Tb.N (3.2 vs 2.61 U\textsuperscript{-1}) 8 weeks post induction. These differences had resolved at 24 weeks. Female mice induced at 10 weeks of age, or GRKO mice induced at 2 or 4 weeks, displayed no changes in bone mass.

**Conclusion:** These results indicate that cartilage-specific GRKO has no apparent effects on cartilage in postnatal and adult mice. However, tamoxifen may affect bone mass in mature male mice.

**PP 92**

**Low Plasma Adiponectin Levels as a Potential Risk Factor in Patients with Osteonecrosis of the Femoral Head**

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**Background:** Both circulatory impairment and abnormalities in metabolism of the bone and lipid are involved in the complex pathogenesis of nontraumatic osteonecrosis of the femoral head (ONFH). A previous study confirmed that adiponectin predominantly exhibited significant anti-inflammatory and anti-atherosclerotic effects. These indirectly prevent obstruction of blood vessels, which leads to certain ischemic diseases. Furthermore, adiponectin might regulate bone formation and bone remodeling by suppressing osteoclastogenesis.

**Objectives:** Therefore, we sought to assess whether plasma adiponectin levels correlated with the susceptibility to nontraumatic ONFH.

**Methods:** Adiponectin levels were measured in nontraumatic ONFH (n=120), traumatic ONFH (n=45), osteoarthritis (n=35) and healthy control subjects (n=120). Other potential influencing factors, such as plasma low-density lipoprotein, high-density lipoprotein, apolipoprotein A1, apolipoprotein B, total cholesterol, triglycerides and C-reactive protein, were also measured by routine methods.

**Results:** Nontraumatic ONFH patients had significantly lower plasma levels of adiponectin than those in the control group (7.14±3.53 vs 10.93±3.41 µg ml\textsuperscript{-1}, P<0.001). Serum adiponectin levels were positively correlated with HDL-cholesterol (r=0.28, P<0.001) and age (r=0.15, P=0.01), yet negatively correlated with body mass index (BMI) (r=−0.70, P<0.001), triglycerides (r=−0.55, P<0.001) and plasma C-reactive protein (r=−0.634, P<0.001). No correlation was seen with LDL-cholesterol (LDL-C) (r=−0.087, P=0.569). There was a significant association between low plasma adiponectin levels and the presence of nontraumatic ONFH with bivariate correlation analysis (r=0.498, P<0.001).

**Conclusions:** Low adiponectin levels are significantly associated with the risk of nontraumatic ONFH. Therefore, this biomarker may be useful to assess the potential risk of nontraumatic ONFH.

**PP 93**

**Glucocorticoid Receptor Deletion in Cartilage Delayed Fracture Healing**

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**Objective:** Although long-term use of glucocorticoids (GC) increases the risk of fracture and hinders fracture repair, the mechanisms by which endogenous GC exert their receptor-mediated effects on fracture healing are poorly understood. In this study, we used a tibial fracture model in tamoxifen-inducible, cartilage-specific glucocorticoid receptor knockout (GRKO) mice to elucidate the role of endogenous GC in fracture healing.

**Methods:** GR\textsubscript{fox/flox} mice were crossed with Col2a1-CreER\textsuperscript{T2} mice to generate tamoxifen-inducible Col2-GRKO mice, in which the cre recombinase is expressed under the control of the type I collagen (Col2) promoter. An open mid-diaphyseal tibial fracture was generated in 10-week-old male mice and fixed by needle insertion. GRKO was induced by tamoxifen (1 mg per 10 g BW per day) on days 1, 3 and 5 post fracture. Healing was monitored by weekly X-rays and by histology and micro-CT at the end point.

**Results:** Compared to WT mice, cartilage callus volume (CV) was significantly increased in GRKO mice on day 7 post fracture (P=0.03). After 14 days, CV declined in both WT and GRKO mice but remained higher in GRKO animals (P=0.06), suggesting a delay in bone remodelling. Micro-CT of the previous fracture area, obtained on day 28 post fracture, revealed significantly increased BV/TV in WT compared to GRKO mice (P=0.02).

**Conclusion:** In summary, our results indicate that cartilage-specific GRKO results in impaired fracture healing, pointing to a role of GCs in endochondral bone formation and fracture repair.
Objective: To investigate the effects of low-dose glucocorticoid (LGC) short-term therapy on bone metabolism in early-activity rheumatoid arthritis (RA) patients.

Methods: 48 postmenopausal women with RA disease activity at early stage were randomly divided into the low-dose glucocorticoid therapy group (LGC group) and non-glucocorticoid therapy group (NGC group). Biomedical markers including inflammation markers (IL-6, IL-1α, TNF-α, CRP and ESR), bone metabolism markers (bone formation markers: BGP, BAP and PICP; bone resorption markers: uDPD and CTX) and bone mass density (BMD) were measured for both RA groups at baseline and in a 3-month trial. The results were compared with those of 50 healthy controls at baseline.

Results: (1) The rate of low bone mass and osteoporosis were higher in postmenopausal female patients with early-activity RA than in healthy controls (P<0.05). (2) In both RA groups, inflammation markers (IL-6, IL-1α and TNF-α) were significantly higher (P<0.01), bone formation markers (BGP, BAP and PICP) were significantly lower (P<0.05), and bone resorption markers (uDPD and CTX) were significantly higher (P<0.05) compared with healthy controls at baseline. (3) There was no significant difference observed for all measured indexes (P>0.05) between LGC and NGC groups at baseline. At the 3-month trial, inflammation markers (IL-6, IL-1α, CRP and ESR) decreased dramatically in both the NGC group (P<0.05) and LGC group (P<0.01), while IL-1α level was even lower in the LGC group than in the NGC group (P<0.05). The levels of bone formation markers were higher, while the levels of bone resorption markers were lower in the LGC group than in the NGC group, but no significant differences were found (P>0.05). At the 3-month trial, BMD increased slightly in both groups, but no significant differences compared with baseline were found (P>0.05), either.

Conclusions: (1) The rate of low bone mass and osteoporosis were higher in postmenopausal female patients of early-activity RA than in healthy controls. (2) LGC short-term therapy could increase bone formation, inhibit bone resorption and reduce bone loss by alleviating inflammation responses in early RA patients. (3) There was no negative effect of LGC short-term therapy on bone metabolism and there was no increase in the rate of osteoporosis in early-activity RA patients.

Objective: In this study, we investigated whether the scaffolds poly lactic-co-glycolic acid/tricalcium phosphate (PLGA/TCP) and PLGA/TCP/Icaritin promoted bone defect repair in rabbits with SAON by recruiting mesenchymal stem cells (MSCs).

Methods: Bone marrow MSCs were aspirated from iliac crest in rabbits before steroid treatment. Wound-healing assay was performed to evaluate the effect of Icaritin on MSC migration. Modified superparamagnetic iron oxide (SPIO; SPIO@SiO$_2$-NH$_2$) labeling efficiency was evaluated by Prussian blue staining. The labeled MSCs’ viability was evaluated by MTT method and the osteogenic and adipogenic differentiation potentials were evaluated by Alizarin Red S staining and Oil red O staining. Twelve 28-week-old male New Zealand white rabbits were used in this study and SAON model establishment followed standard protocol. Two weeks after SAON establishment, PLGA/TCP and PLGA/TCP/Icaritin scaffolds were implanted into the bone tunnel after core decompression, where the initial necrotic bone lesions were formed in rabbits with SAON, immediately followed by injection of SPIO@SiO$_2$-NH$_2$-labeled MSCs into the marrow cavity 20 mm from the defect region. After sample collection and decalcification, the sections of specimens were prepared for Prussian blue and nuclear fast red staining and then microscopic analysis.

Results: Wound-healing assay showed that Icaritin promoted the migration of MSCs. Both PLGA/TCP and PLGA/TCP/Icaritin promoted bone defect repair in rabbits with SAON by recruiting mesenchymal stem cells (MSCs).
the PLGA/TCP/icarin group, similar results as seen in the PLGA/TCP group were found, and there were more labeled cells appearing in the scaffold in the PLGA/TCP/icarin group compared with that in the PLGA/TCP group.

**Conclusion:** Our *in vitro* and *in vivo* studies showed that both PLGA/TCP and PLGA/TCP icarin accelerated bone defect repair in rabbits with SAON through enhanced local MSC homing.

**PP 97**

**Part Two: Relationship between Platelet-Derived Microparticle and Expression of GPIIb/IIa and CD62P in Patients with Ischemic Osteonecrosis of the Femoral Head and the Clinical Effect of Traditional Chinese Medicine**

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**Objective:** (1) To detect the relationship between plasma platelet-derived microparticle (PMP), activation ratio of glycoproteinIIb/IIa (PAC-1) and P-selection (CD62P) in patients with ischemic osteonecrosis of the femoral head (INFH), and to investigate the function and clinical significance of the activation of PMP PAC-1 and CD62P in patients with INFH. (2) To detect the change in the number of PMP and the activation ratio of PAC-1 and CD62P in INFH before and after treatment with traditional Chinese medicine, and to investigate the function and influence of traditional Chinese medicine on platelet activation in INFH patients.

**Methods:** The randomized, double-blind and placebo-controlled study methods were used in 40 INFH patients, who were divided into the Huogu capsule treatment group (group A) and placebo-controlled treatment group (group B) with 20 patients, respectively. Twenty healthy volunteers were selected as group C. Flow cytometry was used to measure the quantity of PMP and activation ratio of PAC-1 and CD62P. Blood-lipid indicators (such as total cholesterol, triglycerides, high-density lipoprotein, low-density lipoprotein, apolipoprotein B and apolipoprotein A1), coagulation indicators (including prothrombin time, activated partial thromboplastin time, prothrombin international normalized ratio), hemorheology index and plasma C-reactive protein concentration were measured by automatic-biochemical analyzer.

**Results:** The quantity of PMP, activation ratio of PAC-1 and CD62P, and the index of hemorheology showed a significantly positive correlation (r=0.34, P<0.01), and there was a significant association between high plasma PMP quantity and the development of INFH (r=0.28, P=0.03, r=0.61, P<0.01, and r=0.15, P=0.04, respectively).

**Conclusion:** The levels of PMP, PAC-1 and CD62P can be taken as the specificity laboratory indices for clinical diagnosis of INFH. The quantity of PMP, activation ratio of PAC-1 and CD62P, and the index of hemorheology showed marked improvement by using traditional Chinese medicine in INFH patients.

**PP 98**

**Potential Therapeutic Targets of Alendronate and PTH for Glucocorticoid-Induced Bone Loss**

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**Introduction:** Glucocorticoid (GC) excess decreases bone mineralization and microarchitecture and leads to reduced bone strength. Both anabolic agent parathyroid hormone (PTH) and anti-resorptive agent alendronate (ALN) are used to prevent and treat GC-induced bone loss, yet these bone active agents alter bone turnover by very different mechanisms. Our study objective was to determine how PTH and ALN alter bone quality following GC excess.

**Method:** Nine-month-old SD male rats were treated with methylprednisolone (5 mg/kg) or placebo (CON). Other two groups of GC-treated rats had either PTH (5 μg/kg) or ALN (5 μg/kg) intervention. Bone quality and quantity measurements include dual X-ray absorptiometry (DEXA) for the degree of bone mineralization, histomorphometry for bone microarchitecture and biochemistry for bone turnover. Osteoblasts were isolated from newborn rats cultured *in vitro*. Cells were treated with (1) CON: placebo; (2) GC: Dexamethasone 10^-5; (3) ALN: Alendronate 10^-7; (4) PTH: human recombinant parathyroid hormone 10^-7; (5) GC+ALN: Dexamethasone 10^-5+ALN 10^-7; (6) GC+PTH: Dexamethasone 10^-5+PTH 10^-7. Osteoblast proliferation and osteogenic activity were identified by MTT and alkaline phosphatase detection; calcium nodules were observed by Alizarin red staining, and real-time PCR was performed to monitor the expression of several key genes regulating bone formation and mineralization as FGF23 and SOST.

**Results:** Spine and femur BMD was reduced after GC treatment, and it was restored to CON level with GC+ALN and GC+PTH at the site of femur and spine separately. Compared to the CON, GC treatment decreased trabecular bone volume, bone formation rate, mineral apposition rate and serum BGP, but increased osteoclast surface and serum TRAP5b. GC+PTH increased and GC+ALN restored trabecular bone volume to the CON levels. GC+PTH increased bone formation rate and serum BGP compared to GC, while GC+ALN increased mineral apposition rate and reduced osteoclast surface and serum TRAP5b. GC+PTH and GC+ALN promoted osteoblast MTX and AKP to the CON levels, but PTH significantly depressed the calcium nodules, whereas ALN increased it. Real-time-PCR revealed that SOST and FGF23 were upregulated by GC treatment, but SOST gene was downregulated after GC+PTH treatment, while SOST and FGF23 genes were both down-regulated by GC+ALN treatment.

**Conclusion:** GC excess increased expression of genes that inhibit osteoblast activity and genes that inhibit mineralization and that were associated with reduced bone formation and mineralization.
Poster abstracts

bone volume. The addition of both PTH and ALN improved bone mass and bone strength during concurrent GC treatment, with PTH lowering expression of SOST and increasing bone formation, while ALN lowered the expression of SOST and FGF23 and reversed the deterioration of bone mineralization induced by GC excess.

PP 99
The Associations of Serum Serotonin with Bone Traits are Age- and Gender-specific
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Serotonin plays a potential role in bone metabolism. But the results of current basic studies were controversial and human studies directly addressing the skeletal effect of circulating serotonin are few. **Objective:** This study aimed to investigate the associations between serum serotonin and bone traits at multiple sites in women and men.

**Subjects and Methods:** Subjects were part of the CALEX-family study and comprised 235 young women, 121 premenopausal women, 124 postmenopausal women and 168 men. Body composition was assessed using DXA, as was areal bone mineral density (aBMD) of the spine, femur and whole body. In addition, pQCT was used to determine bone properties at the tibial midshaft and distal radius. Fasting serum serotonin concentration was assessed using a competitive enzyme-linked immunosorbent assay.

**Results:** Serum serotonin declined with advancing age both in females and in males (all \( P<0.01 \)). Serotonin was negatively correlated with weight, BMI, lean and fat mass in women (\( r=−0.22 \) to \( −0.39 \), all \( P<0.001 \)), but positively with height and lean mass in men (all \( P<0.01 \)). In the premenopausal women, serotonin was negatively correlated with lumbar spine aBMD (\( r=−0.23 \), \( P<0.05 \)) but the statistical significance disappeared after adjustment for weight. Conversely, in postmenopausal women, serotonin was positively correlated with whole body and femur aBMD, as well as with distal radius bone mineral content and volumetric BMD (\( r=0.20−0.30 \), all \( P<0.05 \)), and these associations remained significant after adjustment for weight. In men, no significant associations were found between serotonin and bone traits.

**Conclusion:** Serum serotonin is positively associated with bone traits in postmenopausal women, but not in premenopausal women or men. This partially supports the idea of circulating serotonin playing a role in the regulation of bone metabolism, but also indicates the importance of gender and age-specific factors.