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1

Combination of Tanshinol and Dickkopf-1 Antibody to Stimulate Osteogenic Differentiation of Rats Marrow Stromal Cells through Wnt/β-catenin Pathway

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Aim: Wnt signaling is known as a pathway in the proliferation, migration and differentiation of bone cells. One inhibitor of the Wnt is Dickkopf family Dickkopf-1 (DKK-1). Glucocorticoid (GC) is a strong inducer of DKK1. Our previous study found tanshinol could prevent GC-induced bone loss in rats through stimulating osteoblast bone formation and marrow stromal cells (MSCs) differentiation to osteoblasts, but the underlying mechanism is not very clear. The purpose of this study is to find whether stimulated Wnt signaling by tanshinol is mediated by inhibiting DKK1 in rat MSCs *in vivo*.
Methods: Isolated from 1-month-old SD rats, MSCs were cultured, purified for three passages through labeling double fluorescent marker of CD45R and CD90.1 and identifying the purity of MSCs with flow cytometry. The purified MSCs were treated with vehicle, DKK-1 antibody, different concentration of tanshinol and tanshinol combined with DKK-1 antibody, respectively. The activity of alkaline phosphatase and calcification nodules formed by MSCs were measured. The mRNA expression of DKK-1 and β-catenin was detected by RT-PCR, and the protein expression of DKK-1 and β-catenin was detected by western blot.
Results: For tanshinol-treated group, ALP activity and nodule bone formation in MSCs were found to be increased with the dose of tanshinol from 5×10^{-7} to 5×10^{-6} mol l^{-1} on the 9th and 15th days ($P<0.05$), and this effect was similar to DKK-1 antibody (0.1 mg l^{-1}) on the 6th day ($P<0.01$). The combination of 5×10^{-8} mol l^{-1} tanshinol and 0.01 mg l^{-1} DKK-1 antibody also increased the ALP activity. MSCs could express the DKK-1 and β-catenin mRNA and protein. Compared with vehicle-treated group, tanshinol, DKK-1 antibody, tanshinol combined with DKK-1 antibody increased the expression of β-catenin mRNA and protein and decreased the expression of DKK-1 mRNA and protein.
Conclusions: Tanshinol can stimulate osteogenic differentiation of MSCs by reducing the expression of DKK-1 and enhancing expression of β-catenin, and these effects were similar to those of DKK-1 antibody. It is indicated that tanshinol may stimulate the osteogenic differentiation through Wnt signaling pathway.

2

The Effects of ERβ on Chondrogenic Differentiation of Bone Marrow Mesenchyme Stem Cells

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Objective: To compare the different chondrogenic differentiation potentials of bone marrow mesenchyme stem cells (BMMSCs) between wild-type group and ERβ$^{-/-}$ mice' group *in vitro*.
Methods: Thirty samples of BMMSCs harvested from wild-type and ERβ$^{-/-}$ mice were isolated and cultured. The third passage cells of each group were subjected to FACS (flow cytometry) to analyze cell phenotype as CD29, CD44, CD105, CD34, CD45 and CD11b. The growth curve of the cells in the two groups sample were detected by CCK-8 (Cell Counting Kit-8) staining. The third passage cells were induced to differentiate into chondrocytes by Des-serum-HG-DMEM containing 10 ng ml^{-1} TGF-β1, 100 nmol l^{-1} Decaesadril and 50 μg ml^{-1} antiscorbic acid. Being induced for 21 days, type-I collagen was detected by immunohistochemical analysis and RT-PCR.
Results: The specific MSCs markers CD29, CD4 and CD105 were stained positively and the specific markers of hematopoietic stem cell of CD34, CD45 and CD11b were negative on MSCs of C57 and ERβ$^{-/-}$ mice. Type-I collagen was detected to be positive in both C57 and ERβ$^{-/-}$ mouse' cells after induced for 21 days. The differentiation rates of ERβ$^{-/-}$ group were 42.61 (±6.72) % compared with that of C57 group 56.30 (±5.25) % ($P<0.05$). The cells of C57 group had higher type-I collagen mRNA expression than that of ERβ$^{-/-}$ ($P<0.01$).
Conclusions: MSCs isolated from both wild-type and ERβ$^{-/-}$ mice' could be successfully differentiated into chondrocytes. The *ER*β gene appears to have a role in the chondrogenic differentiation of MSCs.

3

Simulation Study on Bone Perfusion with Different BMD

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Objective: Previous DCE-MRI study on bone perfusion indicated a reduced blood perfusion in subjects with lower bone mineral density (BMD). However, details of the perfusion process, such as blood perfusion volume in different marrow contents, cannot be reflected by the *in vivo* study. The objective of this study is to investigate the perfusion process of cancellous bone in subjects of varying BMD by simulation, which will deepen the understanding on the bone perfusion in osteoporotic bone.
Methods: Two female subjects were involved, where one (70 years) was with normal BMD and the other (65 years) was an osteoporotic patient. Quantitative CT was acquired at the L3 lumbar spine to obtain BMD and trabecular structure. ^{1}H MR spectroscopy was performed to obtain the fat content of the bone marrow. Finite element analysis model was adopted to simulate the perfusion process. In the model, trabucular bone constructed the framework and the inter-trabecular space was filled by red and yellow marrow, respectively. Perfusion in the bone marrow was simulated under the condition with a modeled arterial input function. The perfusion was simulated for 500 s. Quantitative parameters, maximum enhancement and maximum slope, were used to quantify the perfusion characteristic curve.
Results: The two investigated vertebra had 27.76 and 8.62% trabecular bone and 52.61 and 75.78% fat content, respectively. The simulation results showed a reduced perfusion as a whole in the osteoporotic bone. Further, the perfusion was also reduced in both red and yellow marrow in the osteoporotic bone. In addition, the perfusion curve showed an obvious wash-out immediately after the wash-in phase in the bone with normal BMD, while it showed an increasing trend to a saturation level. In summary, first, the reduced perfusion in osteoporotic bone repeated previous findings, which validate the simulation method. Second, the perfusion was reduced not only in the bone as a whole but also in respective marrow contents, that is, red and yellow marrow. It also indicated that the perfusion process not only happened in red marrow but also in the yellow marrow. Third and interestingly, the perfusion showed a different pattern in osteoporotic bone.
Conclusions: The osteoporotic bone showed an obvious delayed wash-out phase compared with that in the normal bone. As the perfusion is the process for the nutrition exchange of the tissue, whether its pattern change could cause the bone loss is worthy of further investigation.

4

Mutational Survey of the PHEX Gene in 10 Unrelated Chinese Families with X-Linked Hypophosphatemic Rickets/Osteomaracia

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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: Ten 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 6 novel mutations in the 10 unrelated families. 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. In family 2: the proband (II1, 4-year-old girl) and her mother (I2, 27-year-old female) carried a novel missense mutation c.1751A>C in exon 17, resulting in p.H584P. In family 3: the proband (II1, 43-year-old female) and her mother (I2, 73-year-old female) carried a novel nonsense mutation c.1332G>A in exon 12, resulting in p.W444X. 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. 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. 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. 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. In family 8: the proband (III1, 4-year-old boy) and his mother (II2, 33-year-old female) carried a splicing mutation c.1646-2A>T in intron 15. In family 9: the proband (II1, 14-year-old boy) and his mother (I2, 37-year-old female) carried a splicing mutation c.1174-1G>A in intron 10. In family 10: the proband (II1, 40-year-old female) and her mother (I2, 59-year-old female) carried a deletion mutation c.1694delA in exon 16, resulting in p.Y565Ffsx5. 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 (III1, 3-year-old boy) carried a deletion mutation c.2154_2169delinsA in exon 22, resulting in p.N718_N723delinsK. 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 were useful to understand the genetic basis of Chinese patients with XLH.

5

Ultrastructure of Bone Mineral Formed at the Defect in Rats with Streptozotocin Diabetes

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Aim: To study the ultrastructure of the mineral that bone formed at the defect in mature streptozotocin diabetes rats.
Materials and methods: To study the reparative regeneration of bone tissue in diabetes, we carried out an experiment on 210 white rats. Rats of specified age group were divided into three groups of 35 animals in each. The first control group consisted of animals without defects. The second group rats

contained perforated defect in the proximal metaphysis of the tibia (defect without diabetes). The third group consisted of streptozotocin diabetes with perforated defect.
Results: Application of defect in tibia of streptozotocin diabetic rats showed the signs of slowing down the formation of bone mineral: comparing with control animals at 7-day microtexture coefficient was lower at 8.44%. Further dimensions of unit cells in regenerate bone mineral were greater than control in the period from 15th to 90th days of observation: along the axis A—at 0.37, 0.31, 0.19 and 0.10%, and along the axis C—at 0.38, 0.37, 0.30 and 0.17%. Value C/A was not significantly changed. Application of defect on the edge of diaphysis of tibia during the period from 7th to 60th days resulted in an increase in bone regenerate content of calcium carbonate and amorphous calcium phosphate to 28.65, 18.08, 13.36% and 6, 92% ($P>0.05$) and 17.16, 25.48, 20.16 and 6.92%, respectively.
Conclusions: The processes of reparative regeneration of bone in mature rats with streptozotocin diabetes are characterized by gross violations of the ultrastructure of the mineral. As the modified unit cells are more certain than changes in the size of crystallites, one can assume that the defective process of mineralization occurs in the first phase of this process.

6

The Effects of Insulin on Bone Mass and Turnover and its Related Molecular Mechanism in STZ-Induced Diabetic Rats

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Objective: To study the effects of insulin on bone turnover and its related molecular mechanism in STZ-induced diabetic rats.
Methods: Of 36 male SD rats studied, 24 were made diabetic by intravenous injection of streptozocin. Twenty-four diabetic rats were divided into group DM and group INS. After killing on day 56, serum Ca, P, ALP and OC were measured. The left tibia was dissected for bone histomorphometry analysis. Right femur and lumbar vertebrae (L1–L4) were reserved for BMD test. The right tibia was separated for the study of bone tissue, RANKL/OPG, Cbfa1, OSX, OC and PPARg2 mRNA level, which was performed by real-time quantitative reverse transcription PCR assays.
Results: A low-turnover osteopenia was observed in diabetic rats with decreased BMD, reduced serum OC level, decreased trabecular mass and reduced bone label surface and bone formation rate. All these abnormalities were partly or completely normalized by insulin. The RANKL/OPG, OSX, Cbfa1 and OC mRNA expressions were declined in diabetic bone, compared with the control. OSX, Cbfa1 and OC mRNA levels were reversed by insulin in diabetic tibia, while RANKL/OPG mRNA showed no change. The expression of PPARg2 mRNA, a marker for adipocytes, showed an increase in the diabetic bone, compared with the control. With insulin treatment, it was decreased with reduction in marrow adipocity compared with diabetic rats.
Conclusion: A low-turnover osteopenia was observed in STZ-induced diabetic rats with significant decrease of both osteoclastic marker (RANKL/OPG) and osteoblastic marker (OSX, Cbfa1, OC) mRNA level in tibia. Insulin can increase bone mass by stimulating bone formation, as demonstrated by bone dynamic parameter and molecular biochemistry study. The study indicated insulin may not influence osteoclastic activity of diabetic rats. We also conclude that selection of adipogenesis over osteoblastogenesis may be responsible for diabetic bone loss, and that insulin can increase diabetic bone mass by reversing this change.

7

Desmethylicaritin Inhibits Adipogenesis Via Wnt/β-Catenin Signaling Pathway in 3T3-L1 Cells

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Aims: Epimedium-derived flavonoids (EFs) were able to inhibit adipogenesis. Recently, we found that desmethylicaritin was one of the metabolites of EF in serum. In this study, we investigated the inhibitory effects of desmethylicaritin on adipogenesis and the underlying mechanism.
Methods: After reaching confluence, 3T3-L1 cells were induced with differentiation medium (designated as day 0). Desmethylicaritin was added to the medium over the full course of differentiation. Oil Red O staining was used to investigate the effects of desmethylicaritin on adipogenesis at day 8. Real-time PCR was performed to quantify the mRNA expression of CCAAT/enhancer-binding protein delta (Cebpd) and beta (Cebpb) at 1, 3 and 8 h, CCAAT/enhancer-binding protein alpha (Cebpa), peroxisome proliferator-activted receptor gamma (Pparg), adipocyte lipid-binding protein (Fabp4) and lipoprotein lipase (Lpl), Wnt10b and β-catenin at days 2, 4, 6 and 8. Immunofluorescence was performed to observe the nuclear translocation of β-catenin at day 2. All quantitative data were presented as means±s.d. of three experiments. One-way analysis of variance followed by Tukey *post hoc* test were used to assess statistical significance at $P<0.05$ using the SPSS 17.0 software.
Results: Different concentrations of desmethylicaritin in 0.1, 1 and 10 μM all significantly decreased the amount of lipid droplets in 3T3-L1 cells, when compared with adipocyte control ($P<0.05$ for all), suggesting that desmethylicaritin could reduce the adipogenesis in 3T3-L1 cells. Desmethylicaritin did not affect Cebpd and Cebpb mRNA expressions, and the mitotic clonal expansion in the early phase of adipogenesis. In contrast, 10 μM of desmethylicaritin significantly decreased the mRNA expression of the following adipogenic transcription factors, Cebpa and Pparg, and adipocyte-specific genes, *Fabp4* and *Lpl*, compared with that in the adipogenic-induced cells without desmethylicaritin treatment ($P<0.05$ for all at days 4, 6 and 8). Further, desmethylicaritin upregulated the mRNA expression of Wnt10b, which was decreased by adipogenic induction, but did not affect the mRNA expression of β-catenin

during the adipogenesis process. However, immunofluorescence results showed that desmethylicaritin increased nuclear translocation of β-catenin at day 2.
Conclusions: The present results demonstrated that desmethylicaritin inhibited adipogenesis by downregulating the expression of Cebpa and Pparg, which may be mediated via Wnt/β-catenin pathway.

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Effects of Drynaria Total Flavonoid on Osteogenic Differentiation of SD Rat Bone Mesenchymal Stem Cells at Different Glucose Concentrations

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Objective: To mimic the *in vivo* metabolism of glucose, to study the effects of drynari total flavonoid on osteogenic differentiation of bone mesenchymal stem cells (BMSCs) at different glucose concentrations.
Methods: BMSCs of SD rats were cultivated and induced to differentiate into osteoblasts under *in vitro* conditions. The whole bone marrow culture method was used to isolate and purify BMSCs from SD rats. BMSCs were treated with blank control (Group A), blank control (Group B), low glucose classical induction group, high glucose classical induction group, low glucose with drynaria total flavonoid group and high glucose with drynaria total flavonoid group. Then the osteogenesis indices were detected to evaluate the effect of drynaria total flavonoid on the BMSC osteogenic differentiation potential at different glucose concentrations.
Results: Compared with the low glucose group, the high glucose group showed an inhibitory effect on BMSC osteogenic differentiation. ALP activity, ALP staining positive percent, calcium nodules and type I collagen expression were lower in the high glucose induction group than in the low glucose induction group; and that of high glucose drynaria total flavonoid group were lower than in the low glucose drynaria total flavonoid group ($P<0.05$). Drynaria total flavonoid increased the expression of BMSC osteogenic differentiation index more effectively than the classical medicine. The expression of osteogenesis index in low glucose drynaria total flavonoid group was higher than in the low glucose induction group, and the high glucose drynaria total flavonoid group was higher than in the high glucose induction group ($P<0.05$). The expression of AGEs had statistical differences between different glucose concentration groups. AGEs increased with a higher glucose concentration. The concentrations of AGEs had a significant negative correlation with the expression of type I collagen.
Conclusion: Under the condition of high glucose concentration, the inhibitory effect on rat BMSCs as manifested by the deposition of AGEs expression downregulates the expression of type I collagen and then suppresses the process of bone matrix mineralization. Drynaria total flavonoid may promote osteogenic differentiation of BMSCs and relieve the inhibitory effect of high glucose concentration at $100\,\mu g\,ml^{-1}$. Thus, drynaria total flavonoid may provide a potential therapy for diabetic osteoporosis.

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FGFR3 Gene G380R Mutation in A Chinese Patient With ACH and Review of Related Literatures

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Aims: Achondroplasia (ACH) is the most common form of short-limbed dwarfism in human beings, with an estimated prevalence between 1/15 000 and 1/77 000. It is inherited as an autosomal dominant trait with essentially complete penetrance, and is characterized by short stature caused by rhizomelic shortening of the limbs, characteristic facies with frontal bossing and midface hypoplasia, exaggerated lumbar lordosis, limitation of elbow extension, genu varum and trident hand. The gene for ACH has been identified to be the fibroblast growth factor 3 (*FGFR3*), which has been mapped on human chromosome 4p16.3, contains 19 exons spanning 16.5 kb and encodes a membrane-spanning tyrosine kinase receptor of 806 amino acids. In present study, we analyzed a Chinese family with autosomal dominant ACH, and identified a *de novo* mutation of *FGFR3* gene. Meanwhile, in order to improve the cognitive and diagnostic abilities for ACH, we did a review of the related literatures.
Methods: We reported one patient who exhibited typical features of ACH. We used PCR to analyze the mutation and performed gene sequencing among the proband, other normal family members and the control group which included 250 volunteers (male: 125, female: 125) without ACH. Radiological investigations were carried out simultaneously.
Results: A recurrent missense mutation of exon 9 in the proband was found. It was a heterozygous G-to-A transition at c.1138, which resulted in a glycine-to-arginine substitution at p.380. We did not detect *FGFR3* gene mutations in his parents, other family members and 250 healthy volunteers. This missense mutation was a *de novo* mutation in this family.
Conclusions: We found that the FGFR3 gene mutation: p.G380R was a *de novo* mutation, which was responsible for ACH in the proband. It is possible that the FGFR3 G380R amino-acid substitution is the only mutation that causes the ACH phenotype. Our finding was useful to understand the genetic basis of Chinese patients with ACH and may contribute to the early molecular diagnosis and treatment of ACH.

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Netrin-1 Inhibits the Osteoblastic Differentiation

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Objective: Netrin-1 belongs to a family of laminin-related secreted proteins that was discovered initially in the brain. It has crucial roles in nervous system development. The expression and functions of netrin-1 have been identified in many

tissues. However, the role of netrin-1 in osteoblastic differentiation has not been well elucidated. We herein report the role of netrin-1 in osteoblastic differentiation.
Materials and methods: To investigate the functions of netrin-1 on osteoblastic differentiation, we examined the effects of netrin-1 on alkaline phosphatase (ALP) activity, gene expression of osteoblastic markers (ALP, osteocalcin (OCN), type I collagen (Col I)) and osteoblastic transcriptional factors (Runx2, Osterix (OSX)) in mouse preosteoblastic cell line MC3T3-E1. Mineralization of the extracellular matrix was also assayed. In addition to *in vitro* assays, we investigated the expression of netrin-1 in bone tissue.
Results: The expression of netrin-1 and its receptors was detected in preosteoblastic MC3T3-E1 cells. Netrin-1 inhibited ALP activity and mRNA expression of ALP, OCN, Runx2 and Osx. Mineralization of extracellular matrix was also inhibited by the treatment of netrin-1. On the other hand, the expression of netrin-1 was detected in the periosteum and hypertropic chondrocytes in a mouse.
Conclusion: Our results indicate that netrin-1 might inhibit osteoblastic differentiation. Considering the expression of it in bone tissues, it might have an important role in bone metabolism. We propose that Netrin-1 might regulate osteoblastic differentiation. We are going to study signal-transduction pathways of these phenomena.

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Salvianolate Stimulates Bone Formation and Increases Bone Mass in SLE Mice

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Recent studies have reported low BMD and an increased risk of fracture among patients with systemic lupus erythematosus (SLE), and combination treatment with glucocorticoid (GC) can accelerate bone loss in SLE patients. This study was to investigate the effects of salvianolate, a total polyphenolic from aqueous extract of Radix Salviae Miltiorrhizae, on bone tissue in a spontaneous SLE mice model by analysis of bone histomorphometry. Fifteen-week-old MRL/lpr mice were treated with vehicle, GC (prednisone 6 mg kg–1 per day by oral gavage), salvianolate (60 mg kg–1 per day intraperitoneal injection) and GC plus salvianolate, respectively, for 12 weeks. MRL/lpr mice had very low bone mass with only 1.6% in trabecular area (Tb.Ar) comparing to wild-type mice, which had 10% in Tb.Ar. GC-treated MRL/lpr mice displayed remarkably decreased bone formation indices such as mineral apposition rate and bone formation rate/bone volume (BFR/BV) but did not differ in bone mass when compared with MRL/lpr mice. Salvianolate treatment in GC-treated MRL/lpr mice increased bone mass to 5.2% (increase of 123% in Tb.Ar, $P<0.01$) accompanied by an increase in bone formation parameters ($P<0.01$) and femur bone biomechanics properties ($P<0.01$) when compared with GC-treated MRL/lpr mice. Salvianolate treatment alone in MRL/lpr mice also increased bone mass to 3.1% (increase of 83% in Tb.Ar, $P<0.05$) accompanied by an increase in BFR/TV ($P<0.01$) and femur bone biomechanics properties ($P<0.01$) when compared with MRL/lpr mice treated with vehicle. The data support further preclinical investigation of salvianolate stimulation of bone formation as a potential therapeutic strategy in the treatment of SLE-related bone loss.

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Association of the Type 2 Diabetes Mellitus and Osteoporosis in Postmenopausal Women with the BMP-4 Gene Polymorphism

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Objective: The aim of this study was to explore the distribution of polymorphisms in the bone morphogenetic proteins-4 (BMP-4) gene and the association of BMP-4 gene polymorphisms with glycometabolism, bone transformation and bone mineral density (BMD) variation in postmenopausal women with type 2 diabetes mellitus (T2DM) and osteoporosis (OP) in the Shanghai region.
Methods: A total of 485 unrelated postmenopausal women of Han nationality in the Shanghai region were recruited and divided into four groups. Group A (OP group, 120 cases), Group B (T2DM group, 108 cases), Group C (T2DM with OP group, 130 cases) and Group D (healthy control group, 127 cases). The BMD of the lumbar spine L2–4 and femoral neck, bone alkaline phosphatase, tartrate-resistant acidphosphatase-5b, glycosylated hemoglobin A1c (HbA1c) and fasting plasma glucose were measured.
Results: In groups A and C for the spine L2–4 and femoral neck, BMD, the HH genotype was significantly higher than hh genotype ($P<0.001$); the Hh genotype was significant higher than hh genotype ($P<0.001$). There was no significant difference in the L2–4 and femoral neck BMD between the BMP-4 HH genotype and Hh genotype in the groups A (P=0.118 and 0.800, respectively) and C (P=0.118 and 0.800, respectively).
Conclusion: BMP-4 genotype might be a susceptible gene of OP in postmenopausal women in Shanghai region, and the h allele is associated with low bone mass. The H allele is associated with high bone mass. The BMP-4 genotype is not associated with glycosylated hemoglobin.

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A Comparison of the Biological Activity of Osteoblast Between Iron Excess and Iron Deficiency

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Aim: To examine the effects of iron excess and iron deficiency on the biological activity of osteoblasts *in vitro*.
Method: Human osteoblast cells (hFOB1.19) were incubated in a medium supplemented with 0–200 μmol l^{-1} ferric ammonium citrate (FAC) and 0–20 μmol l^{-1} deferoxamine. Proliferation of osteoblasts was evaluated by MTT assay. Apoptotic cells were detected by Annexin intervention V/PI staining with a flow cytometer. ALP activity was measured using an ALP viability kit. The number of calcified nodules and mineral area

were evaluated by Von-Kossa staining assay. The expression of Type I collagen (COL1) and osteocalcin (OC) of cultured osteoblasts was detected by RT-PCR and western blot.
Results: FAC decreased the proliferation, ALP activity, mineralization function, and expression of COL1 and OC at both mRNA and protein levels in cultured osteoblasts in a concentration-dependent manner. FAC increased the percentage of apoptotic osteoblasts. These activity indexes were increased by DFO at low concentration, but were decreased at high concentration.
Conclusion: Excessive iron inhibited osteoblast activity in a concentration-dependent manner. Low iron produced a biphasic effect on osteoblasts: mild low iron promoted osteoblast activity, but extremely low iron inhibited osteoblast activity. Osteogenesis was optimal at a certain iron concentration range.

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Primary Study of the Effect of Magnetic Nanoparticle Assemblies on Osteoblast

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Nanomaterials have a very wide range of applications in the biomedical field, such as cellular tag, scaffold for tissue engineering, carriers for drug delivery, contrast agents for medical imaging, magnetic thermotherapy and gene transfection. Among these applications, the interaction between cells and materials is a key issue. Besides intrinsic property of material, aggregation morphology of nanoparticles is also an important factor to influence cellular behaviors. With the development of nanotechnology, the assembly of individual nanoparticles into specific higher-hierarchical structures can now be controlled by many physical, chemical and biological methods. Thus, the morphological effect of nanoparticle assemblies on cellular behavior should be studied.
In this paper, the influence of chain-like magnetic assemblies on osteoblastogenesis was studied. The magnetic chain-like assemblies were fabricated by evaporation-induced assembly of γ-Fe_2O_3 nanoparticles in the presence of a parallel magnetic field. In our experiments, two parameters of assemblies were adjusted: one is concentration of nanoparticles suspension, the other is field intensity. To study the osteoblast formation, adherent stromal cells were cultured in the presence of ascorbic acid-2-phosphate to initiate differentiation. The process of cell adherence, growth and differentiation were observed by light microscope, phase contrast microscope and scanning electron microscopy. At around 10 days, colony-forming unit-fibroblast (CFU-F) appeared. We stained these colonies for alkaline phosphatase using a kit (Sigma) per the manufacturer. In separate cultures, the colonies were stained with Prussian blue to detect cell iron uptake.
The results showed that magnetic nanoparticle assemblies could significantly affect osteoblast differentiation in a nanoparticle concentration and field strength-dependent way. Cells preferred to adhere to assemblies, and cell shape changed with assembly structures. Osteoblasts on magnetic nanoparticle assemblies were elongated and oriented in the direction of assemblies and presented a lot of filopodia with them. In addition, there was little difference in iron uptake in different assembly groups and less iron uptake than natural drying group because of much more stability after assembling, which implies that iron uptake contributes little to the influence of magnetic nanoparticle assemblies on osteoblasts.
Our study provides a reference for better understanding the interaction between magnetic nanoparticle assemblies and osteoblasts. Further research will focus on the most suitable assembly structure for osteoblast differentiation, and external electromagnetic field will be introduced during cell culture. Moreover, deeper regulation mechanism of magnetic nanoparticle assemblies on osteoblast differentiation and function still needs to be explored. It should have potential applications in tissue engineering, osteoporosis prevention, diagnosis and treatment.

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The Effects of Different Doses of Glucocorticoid on Bone Structure in Growing Male Rats

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Objective: Glucocorticoid has strong inhibitory effects on bone growth in young patients. The current study was designed to investigate the dose effects and the mechanism of prednisone on various bone sites by analyses of bone histomorphometry, bone mineral density, biomechanics, bone marrow fat cells and serum markers in growing male rats.
Methods: Forty-two 3-month-old male Sprague–Dawley rats were used in this study. Six rats were selected and killed at the beginning of this study as baseline control. The remaining 36 rats were randomly divided into four groups with nine rats per group. The rats were treated with either vehicle (CON), or prednisone acetate (GC) at a dose of 1.5 mg kg^{-1} per day (GC-L), 3.0 mg kg^{-1} per day (GC-M) or 6.0 mg kg^{-1} per day (GC-H), respectively. All rats were treated by oral gavage for 90 days. Body weight was recorded weekly. All rats were given injections of tetracycline 35 mg kg^{-1} on the 14th, 13th day and calcein 10 mg kg^{-1} on the 4th, 3rd day before killing for the purpose of double *in vivo* labeling. At the end point, rats were killed by cardiac puncture under anesthesia. The blood serum was collected for testing biochemical markers. The left proximal tibial metaphysis, left tibia cross-section (TX) and left distal femoral metaphysis (DFM) were prepared in undecalcified sections for bone histomorphometry. The right tibia was processed in decalcified sections for analysis of fat cells in bone marrow. The right femur and the 5th lumber vertebra (LV5) were used for both bone mineral density and biomechanical measurements.
Results: 1. Compared with vehicle-treated age controls, TRACP-5b was significantly decreased in the rats treated with GC-H ($P<0.05$), while the osteocalcin was significantly decreased in the rats treated with either GC-M or GC-H ($P<0.01$, $P<0.01$, respectively). 2. Compared with the vehicle-treated age controls, no significant changes were observed in the static histomorphometric parameters in the GC-treated rats. However, the rats treated with GC-H showed significant

decrease in all dynamic bone formation parameters, and significant decrease in number of osteoblasts (Ob.N), percent of osteoblast surface (% Ob.Pm), number of osteoclasts (Oc.N) and percent of osteoclast surface (% Oc.Pm). Rats treated with GC showed significant decrease in the thickness of the femur cortical bone, and noticeable cortical bone porosity was seen in the TX of the rats treated with GC-M or GC-H. 3. Compared with the vehicle-treated age controls, the rats treated with GC-H showed significant decrease in BMD and BMC ($P<0.05$, $P<0.05$, respectively) in the right femurs and significant decrease in elastic stiffness, maximum load, breaking load and rigidity coefficient in the femurs ($P<0.01$, <0.01, <0.01 and $P<0.01$, respectively). Significantly decreased in maximum compression load and elastic modulus ($P<0.01$, $P<0.05$, respectively) were also found in the LV5. 4. Compared with the vehicle-treated rats, the rats treated with GC showed significant decrease in the longitudinal growth rate in both femurs and tibiae. Rats treated with GC significantly increased in the percent of adipocyte area in bone marrow.

Conclusions: Long-term use of glucocorticoid significantly inhibited bone growth, bone formation and resorption in cancellous bone, but no cancellous bone mass were decreased. Prednisone acetate inhibited bone formation in cortical bone, but stimulated cortical bone resorption, leading to significant decreased bone mass and increased porosity in cortical bone, and therefore significantly decreased in biomechanical properties in both femur and vertebra bodies.

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Comparison of the effects of Low-Magnitude High-Frequency Vibration and Alendronate on Bone-implant Osseointegration in Osteoporotic Rats

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Objective: To compare the effects of low-magnitude high-frequency (LMHF) whole body vibration and alendronate on bone-implant osseointegration in osteoporotic rats.

Methods: Thirty rats were ovariectomized to induce osteoporosis, and then treated with either LMHF vibration (VIB), or alendronate (ALO), or no treatment (OVX). Another 10 rats were sham operated as Sham control group. Before treatment, hydroxyapatite (HA)-coated titanium implants were inserted into proximal tibiae bilaterally. Both LMHF vibration and alendronate treatment lasted for 8 weeks.

Results: BMD (0.2275±0.0225 g cm^{-2}), maximum push out force (180.35±22.28 N), interfacial shear strength (6.53±0.82 N mm^{-2}), bone-implant contact (69.35±3.79%) and bone area (72.69±1.82) of group VIB were significantly higher than group OVX (0.2019±0.0217 g cm^{-2}, 118.75 N, 4.32±0.6 N mm^{-2}, 51.24±5.36, 45.13±3.60%, respectively; $P<0.05$), but were lower than group ALO (0.2535±0.0362 g cm^{-2}, 224.10±27.59 N, 8.26±0.90 N mm^{-2}, 83.32±6.22, 80.33±2.91%) and Sham (0.2611±0.0279 g cm^{-2}, 245.08±32.65 N, 8.92±1.15 N mm^{-2}, 91.37±4.95, 88.92±5.75%; $P<0.05$). No significant difference was found between ALO and Sham groups.

Conclusion: LMHF whole body vibration enhances bone-implant osseointegration in ovariectomized rats, but was less effective when compared with alendronate.

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Differential Proteomic Analysis on Osteoporotic Rats Stimulated by Low-Level Mechanical Vibration

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Objectives: Low-level mechanical vibration can promote bone formation and prevent osteoporosis. The purpose of this research was to perform proteomic analysis of bone tissue in osteoporotic rats and to identify the differential protein expression under low-level mechanical vibration loading.

Methods: Twelve female Wistar rats, aged 6 months, were randomly divided into the following two groups: daily loading group (DL) and ovariectomized only comparison group (OVX). The postmenopausal osteoporosis model was established by bilateral ovariectomies. Vibration treatment began 1 week after the ovariectomy. Rats in DL were loaded with 35 Hz, 0.25 g low-level mechanical vibrations for 15 min per day, while no treatment for the OVX group. After 8 weeks, all rats were killed and limb bone was harvested and prepared for proteomic analysis. The protein profile of pre- and post-vibration was determined by two-dimensional gel electrophoresis (2-DE) and LC-MS-MS/MS protein identification protocol.

Results: The 2-DE expression maps of the two groups were well matched. Compared with OVX, 27 differentially expressed proteins were obtained, related to signal transduction, osteogenesis, cell proliferation, cytoskeleton rearrangement and energy metabolism. Among them, 25 were upregulated and 2 were downregulated significantly.

Conclusions: We observed that differential proteomic expressions in the osteoporotic rats stimulated by low-level mechanical vibration. These differential proteins could be considered as potential new candidate targets for treating osteoporosis. They may also have an important role in the molecular mechanism of osteogenesis under mechanical vibration loading.

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The Effects of Rosiglitazone on the Secretion of ALP, BMP-2, TGF-β1 from Rat Osteoblast Bone Mesenchymal Stem Cells

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Objective: To observe the effects of varying doses of rosiglitazone on rat bone marrow stromal cells (BMSCs) differentiation into osteoblasts (OB) and the roles of alkaline phosphatase (ALP), bone morphogenetic protein-2 (BMP-2) and transforming growth factor-beta I (TGFβI) BMSCs differentiation.

Methods: To obtain BMSCs from long bone marrow of rats, and breed by using differential time adherent culture method, then to interfere them into osteoblasts with different doses of rosiglitazone (1, 2, 5 or 10 μmol l^{-1}) in the presence of an osteogenic medium. The rate of mineralization was examined by staining of mineralized nodules with Alizarin red S, and the difference in ALP, BMP-2 and TGF-β1 between inter blocks was examined by enzyme linked immunosorbent assay after 21 days of culture.

Results: Compared with the classic group, the activity of ALP, BMP-2 and TGF-β1 of different doses of rosiglitazone concentration (1, 2, 5, 10 μmol l^{-1}) decreased significantly ($P<0.05$). Compared with 1 μmol l^{-1} rosiglitazone group, the rate of mineralization reduced significantly ($P<0.05$), and the decrease of activity of ALP, BMP-2 and TGF-β1 of 2 μmol l^{-1} rosiglitazone group was not significant ($P>0.05$); however, the activity of ALP, BMP-2 and TGF-β1 of 5 μmol l^{-1} rosiglitazone group decreased significantly ($P<0.05$), the same as 10 μmol l^{-1} rosiglitazone group compared with 5 μmol l^{-1} rosiglitazone group.
Conclusion: Rosiglitazone inhibited differentiation of BMSCs into osteoblasts. This suggests that it may decrease bone mass by inhibiting osteoblastic differentiation and promoting adipogenesis differentiation. When the concentration of rosiglitazone was higher, the inhibition was more obvious, and it may be the important potential pathogenesis of rosiglitazone-caused osteoporosis.

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The Effect of a Biphasic Injectable Bone Substitute of the Interface Strength in a Rabbit Knee Prosthesis Model

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Objective: Injectable bone substitute has been used as bone void filler. However, it is unclear if it can stabilize prosthesis in arthroplasty. The aim of the study was to investigate the prosthetic stabilization and tissue integration with or without the use of a biphasic injectable bone substitute in a rabbit knee model.
Materials and methods: Sixteen rabbits were used in the study. Bone marrow cavity in tibia was reamed with a 3.6 mm drill and a round tibia prosthesis stem of 3.5 mm in diameter and 8 mm in length was inserted in the proximal tibia. The rabbits were operated with bilateral prosthesis and the marrow was filled with or without injectable bone substitute (Cerament, Bonesupport, Sweden) consisting of 60% α-calcium sulfate hemihydrate and 40% hydroxyapatite with a radiocontrast agent, iohexol. The rabbits were euthanized after 6 and 12 weeks. Six rabbits in each period were analyzed by a mechanical pull out test and histology. Thereafter, the percentage of bone contact for each cross-section was calculated by measuring the bone contact length divided by total prosthesis length. Micro-CT was used to analyze the total bone volume (1 mm around the prosthesis) from two rabbits in each period.
Results: The interface strength between the prostheses fixed with or without Cerament showed no significant difference at 6 and 12 weeks; however, in the group using Cerament, the pull out strength from 6 to 12 weeks increased by 46% ($P<0.05$), but not the group with the prosthesis without Cerament. Histology showed a homogenous bone/prosthetic contact. The remaining hydroxyapatite particles were integrated in the new-formed bone. Histomophometry and micro-CT showed similar bone contact in percent and bone volume around the prosthesis in the two groups.
Conclusion: In the present study, the bone substitute provided prosthetic stabilization, especially increased the interface strength in later stage. As shown in histology and micro-CT, the proportion of new-formed bone in the interface was similar in the two groups. The new-formed bone in the Cerament group contained hydroxyapatite particles and a different bone quality, which in turn may give additional stability to the prosthetic component. The study suggests that Cerament can be used as bone void filler in prosthetic fixation.

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Femur Metaphysis Bending Test of Rat: Introduction and Validation of a Novel Biomechanical Testing Protocol

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Aim: To validate a novel biomechanical test for femur metaphysis in ovariectomized rats.
Methods: Sixteen 5-month-old female Sprague–Dawley rats were randomly divided into the ovariectomized (OVX) group and the sham-operated (Sham) group. Twelve weeks after operation, examination of femur BMD and histomorphometry of proximal tibiae were performed. Furthermore, biomechanical properties of femur were determined by diaphysis three-point bending test and a novel designed method, named metaphysis bending test. Pearson χ^2-independence tests were performed to analyze the correlation relationships between the biomechanical parameters and BMD and bone mass, respectively.
Results: The femur BMD, bone mass indexes (% Th.Ar, Tb.N, Tb.Th) and biomechanical properties (maximum load, yield load and stiffness) of group OVX were inferior to Sham group ($P<0.05$). In three-point bending test, the mean difference of the maximum load (Fmax), yield load (Fy) between groups OVX and Sham were significant lower than that in metaphysis bending test. A positive correlation between biomechanical parameters and femur BMD or bone mass indexes was observed in both three-point bending and metaphysis bending test. The biomechanical parameters in metaphysis bending test showed a stronger relationship with BMD and bone mass.
Conclusions: A novel biomechanical test, named femur metaphysis bending test, is validated to assess osteoporosis in our study. Femural metaphysis bending test is more sensitive than diaphysis three-point bending test in evaluating the change of biomechanical properties of femur in osteoporotic rats.

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Protection by Tetrahydroxystilbene Glucoside Against Bone Loss Via Inhibition of Oxidative Stress and Bone-Resorbing Mediators

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Objective: Oxidative stress is a pivotal pathogenic factor for bone loss in mice. 2,3,5,4′-Tetrahydr-oxystilbene-2-O-β-D-glucoside (TSG), is a potent antioxidant derived from a Chinese

herb *Polygonum multiflorum* Thunb, exhibits potent antioxidative effects.
Methods: At present study, we used an *in vitro* oxidative stress model induced by hydrogen peroxide (H_2O_2) in MC3T3-E1 cells and an *in vivo* ovariectomy-induced osteoporosis model in mouse to investigate the protective effects of TSG on bone loss and the related mechanisms.
Results: We demonstrated that TSG caused a significant ($P<0.05$) elevation of cell survival, alkaline phosphatase (ALP) staining and activity, calcium deposition, and the mRNA expression of ALP, COL-I and OCN in the presence of H_2O_2. Moreover, TSG decreased the production of intracellular reactive oxygen species, malondialdehyde and osteoclast differentiation inducing factors such as receptor activator of nuclear factor-kB ligand (RANKL) and IL-6 induced by H_2O_2. *In vivo* studies further demonstrated that TSG supplementation at 20 mg kg^{-1} for 3 months caused slight decrease in oxidized glutathione concentration in the blood of ovariectomized mouse ($P<0.05$) and improved bone structural indices, bone mineral density and trabecular thickness in the third lumbar vertebra.
Conclusion: Our study indicates that protection by TSG to bone loss was mediated, at least in part, via inhibition of the release of bone-resorbing mediators and oxidative damage and TSG can be used as an effective remedy in the treatment or prevention of osteoporosis.

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Effects of High Iron Environment on Human Osteoblastic Functions
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Objective: To investigate the effects of high iron environment (ferric ammonium citrate) on osteoblastic functions and OPG/RANKL system *in vitro*.
Methods: Human osteoblast cells (hFOB1.19) were incubated in media supplemented with 0–200 μmol l^{-1} of ferric ammonium citrate (FAC). Proliferation viability of osteoblasts was evaluated by MTT assay at 48 h. Alkaline phosphatase (ALP) activity was measured using ALP viability kit at 10 days. Vonkossa staining assay was used to evaluate mineralized bone nodules at 15 days. The gene and protein expression of OPG and RANKL was detected by RT-PCR and western blot at 48 h after treatment with FAC.
Results: After treatment with FAC for 48 h, FAC significantly inhibited cell proliferation ($P<0.05$) at 50 μmol l^{-1}, which is more obvious at 100 and 200 μmol l^{-1} FAC ($P<0.01$). After treatment with FAC for 10 days, the ALP activity of osteoblasts was significantly suppressed by iron overload dose-dependently ($P<0.01$). The number of mineralized nodules was significantly reduced by FAC at 15 days. In addition, FAC at 100 and 200 μmol l^{-1} significantly increased the mRNA and protein expression of RANKL/OPG ($P<0.01$).
Conclusions: High iron environment not only significantly inhibited cell proliferation, differentiation and mineralization of human osteoblasts, but also increased the mRNA and protein expression of RANKL/OPG. Therefore, iron overload not only inhibited bone formation directly, but also increased bone resorption indirectly in the pathogenesis of osteoporosis.

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The Effect of Drug-Containing Plasma of Chinese Herbal Compound for Tonifying Kidney and Strengthening Spleen on Bcl-2 Family of Proteins of Osteoblasts
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Objective: To study the effect of drug-containing plasma of Chinese herbal compound for tonifying kidney and strengthening spleen on Bcl2 family of proteins of osteoblasts.
Methods: One-day-old SD rats were used to culture osteoblasts, which were divided into three groups. Control group was cultured with the medium of drug-containing plasma of estradiol valerate, observation group was cultured with the medium of drug-containing plasma of Chinese herbal compound for tonifying kidney and strengthening spleen, normal group was cultured with medium of no drug-containing plasma. After 1 week, the Bcl2 family of proteins were examined.
Results: Compared with control group, the expression level of Bcl-2 Bax and Bid in observation group and control group were significantly enhanced ($P<0.05$). Bcl-2/Bax ratio of observation group was higher than control group, and the difference reached statistical significance ($P<0.05$).
Conclusion: Drug-containing plasma of Chinese herbal compound for tonifying kidney and strengthening spleen could regulate the expression of Bcl-2 family proteins, and mainly upregulate antiapoptotic proteins.

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Effects of Kidney Invigoration on mRNA Expression of TGF-β1 of Osteoblastic Cell
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Background: Migu tablet, which is a Chinese drug of kidney invigoration, is effective on preventing and treating osteoporosis, but the concrete mechanism is not so far clear. TGF-β1 is an important cytokine that can regulate bone resorption and formation. The main objective of this experiment is to investigate the relationship between TGF-β1 and the therapeutic mechanism of Migu tablet.
Objective: To investigate the effect of kidney investigation on mRNA expression of TGF-β1 of osteoblastic cell.
Method: A completely randomized controlled study was conducted. Setting: Institute of Bone and Trauma, Union Hospital, Tongji Medical College, Huazhong University of Technology and Science. Physic liquor of Migu tablet was prepared in the Institute of Bone and Trauma, Union Hospital, Tongji Medical College, Huazhong University of Technology and Science. Positive control drug, which was recombinated basic fibroblastic growth factor (rbFGF), was purchased from Beijing Banding Company. Osteoblatic cells of calvaria were isolated

and cultured from newborn fetal Sprague–Dawley rats, and had migu tablet liquor of different concentration and rbFGF added. Twenty-four hours later, *in situ* hybridization of osteoblatic cells was analyzed by a probe that was made by our laboratory. The mean optical density value of positive particles represented the mRNA expression of TGF-β1.
Results: mRNA expression of TGF-β1 in osteoblasts of experimental group was significantly higher than that of the negative control group, as the concentration of migu tablet liquor rised up. However, The optical density value of TGF-β1 in experimental groups that contained migu tablet liquor at concentration of 5000 and 1000 $\mu g\,ml^{-1}$, respectively, had no significant difference with that in positive group ($P>0.05$).
Conclusion: Migu tablet could stimulate the secretion and synthesis of TGF-β1 in osteoblasts, then promote bone formation and inhibit bone resorption. This may be one of the mechanisms by which kidney invigoration prevented and treated osteoporosis.

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Repair of Large Osteochondral Defect by 'Rib-mosaicplasty' in a Rabbit Model: A Trial of Osteochondral Interfacial Regeneration *In Vivo*

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Purpose: This study developed a 'Rib-mosaicplasty' method to repair large osteochondral defect, and the regenerative results of cartilage and osteochondral interface were evaluated in a rabbit model.
Method: Two pieces of costal cartilage were taken from the Japanese white rabbits and sliced into 3-mm segments. Osteochondral defects (Φ=5 mm, h=3 mm) were repaired by the costal cartilage segments with or without mid-cutting longitudinously in a mosaic method, or remained empty (n=4). After 1 and 3 months, the defects were examined grossly by ICRS Macroscopic Score and microscopically by H&E staining and Safranin-O staining.
Results: Both after 1 and 3 months, not only the Macroscopic but also the Visual Histological Score was higher in mosaic group, and highest in the 3-month mosaic group. The biological regeneration of osteochondral interface was also observed.
Discussion: Our results suggested that the osteochondral interface could be regenerated *in vivo* by costal cartilage and native cancellous bone in a rabbit model. As large costal hyaline cartilage store, 'Rib-mosaicplasty' is expected to provide an effective solution for the treatment of cartilage lesion.

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Expression Involved in Insulin Signaling Pathway in the Kidney of Type 2 Diabetic Osteoporosis Rats Model

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Aims: To explore the expression of PI3K, Akt1, Akt2 and NFe in the kidney of Type 2 diabetic osteoporosis rats.
Methods: One hundred Wistar female rats, 2.5–3 months, after 1 week feeding, were randomly divided into four groups: normal control group (NS, n=24), ovariectomized group (NOVX, n=26), type 2 diabetes control group (DS, n=24), type 2 diabetic rats with ovariectomized group (DOVX, n=24). At 4, 8 and 12 weeks after OVX, the kidneys of five rats from every group were separated after anesthesia. The RNA of renal tissue was extracted from the kidney. The mRNA expression of PI3K, Akt1, Akt2 and NFTh was detected by RT-PCR.
Results: 1. *The expression level of PI3K*: At 4 weeks after ovariectomed, the expression of PI3K in DOVX group is much lower than that in DS group ($P<0.05$). At 8 weeks, the expression of PI3K in DOVX group is much higher than that in DS and NOVX groups ($P<0.05$). At 12 weeks, the expression of PI3K in DOVX group is much lower than that in DS and NOVX groups ($P<0.05$). 2. *The expression level of Akt1*: At 4 weeks after ovariectomed, the expression of Akt1 in DOVX group is significantly lower than that in DS ($P<0.05$). At 8 weeks, the expression of Akt1 in DOVX group is much lower than that in NOVX group ($P<0.05$). At 12 weeks, the expression of Akt1 in DOVX group is lower than that in DS group ($P<0.05$). 3. *The expression level of Akt2:* At 4 weeks after OVX, the expression of Akt2 in DOVX group is much lower than that in DS and NOVX groups ($P<0.05$). At 12 weeks, the expression of Akt2 in DOVX group is much lower than that in NOVX group ($P<0.05$). 4. *The expression level of NFTh:* At 4 weeks after OVX, the expression of NFth in NOVX group is significantly higher than that in NS group ($P<0.05$). At 8 weeks, the expression of NF0 in DOVX group is much lower than that in NOVX group ($P<0.05$). At 12 weeks, the expression of NF2 in DOVX group is much lower than that in NOVX group ($P<0.05$).
Conclusion: The expressions of PI3K, Akt1, Akt2 and NFnd in kidney of DOVX group is lower than that in NS, NOVX and DS groups. The results show that the inhibition of the insulin signaling expressions of PI3K, Akt, NFNF in kidney pathway maybe related to the pathogenesis of type 2 diabetes with osteoporosis.

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The Bone Tissue State in the Field of Damage in Senile Period Rats on the Background of Streptozotoc in Diabetes

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Aim: To study the bone tissue state in place of the defect on the background of streptozotocin diabetes in rats mature age.
Methods: A total of 210 white rats were carried out and divided into three groups of 35 animals in each. The first control group consisted of intact animals. The second group consisted of rats, which applied perforated defect in the area proximal metaphysis of the tibia (defect without diabetes). The third group consisted of animals that against streptozotocin diabetes painted perforated defect in the plot.
Results: We found that in animals of experimental groups relative number of cells in the spongy bone substance decreased—from 63.14±0.73 to 61.86±0.64 unit mm^{-2}. Reticulofibrose tissue was determined only from the 7th to 30th

days after surgery and its contents gradually decreased from 64.17±0.82 to 25.42±0.34%. Volume content of spongy bone increased from 7th to 60th days after the application of defect—from 35.83±0.82 to 74.31±0.81%, and on the 90th day decreased to 36 14±0.49%, due to its active compactization. As for the volume content of bone plate, it appeared on the 15th day of the experiment and its content increased from 10.42±0.77 to 63.86±0.49%. This specific number of cells per unit area of trabeculae was smaller than in experiment group, also throughout the period of observation, its provisions pursuant to 6.34, 8.04, 9.91, 9.11 and 9.12%.

Conclusions: Thus, in streptozotocin diabetes in rats period of senile changes there is a significant slowing in the formation of a bone regenerate well and restoration of the structure of bone structures in parareactive area. Amplitude variations, in general, are somewhat lower than in mature rats. This can be explained by the fact that regeneration processes in the group of animals runs in the background as the original (age dependent) as well and a secondary (diabetic) osteoporosis.

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Application in the Improved Parallel Fixture of Four-Point Bending Fatigue Test of Rat Ulna *In Vivo*

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Aims: To establish efficiently the cortical bone microdamage model of rat ulna *in vivo* via using the improved parallel fixture, which can fit the PLD-5010 fatigue damage electronic machine, so as to analyze the mechanism of bone microdamage.

Methods: According to four-point bending principle, fatigue test principle and Hooke's law, and so on, our group improved a parallel fixture that can also fit the PLD-5010 fatigue damage electronic machine used in the fatigue test. The improved fixture can test two rats at the same time, while the former single fixture our research group invented can only test one per time. The fatigue fixture was composed of the loading plate for rats and left/right forelimb loading device *in vivo*, the latter including the upper connecting rod, the upper connecting shaft, linear elastic spring, the main-device of four-point bending test, the lower connecting rod and other components. Seven-month-old female SD rats were randomized into four groups (n=10/group), namely OVX1W (ovariectomized and loaded for 1 week), OVX2W (ovariectomized and loaded for 2 weeks), SHAM1W (sham-operated and loaded for 1 week) and SHAM2W (sham-operated and loaded for 2 weeks). Loads on the right ulna were applied *in vivo* at 0.0555 N g^{-1} of body weight maximum, load for 10 000 cycles at 4 Hz, 3 days per week for 1 or 2 weeks using improved parallel four-point bending fixture and PLD-5010 fatigue damage test machine. After fatigue load, the rats were killed, respectively, and the right ulnae were bulk stained in 1% basic fuchsin and embedded in methylmethacrylate. The mounted bone slices were used to measure microcrack parameters and analyzed under the Leica microscope.

Results: Compared with the previous single fixture, the improved one has double time increased the experimental efficiency. The microdamage could be observed in each sample and microcrack was the most usual damaged type. Significant difference of Cr.Le, Cr.N, Cr.Dn and Cr.SDn was observed between OVX group and SHAM group ($P<0.05$). Significant difference of Cr.Le and Cr.SDn was observed between OVX1W group and OVX2W group ($P<0.05$). However, Cr.N and Cr.Dn showed no significant differences between OVX1W group and OVX2W group ($P>0.05$). All these parameters showed no significant difference between SHAM1W group and SHAM2W group ($P>0.05$).

Conclusions: The cortical bone microdamage model has been successfully and efficiently established by using the PLD-5010 fatigue damage test machine and improved parallel four-point bending fixture. A platform was built more rapidly for the establishment of the microdamage model. The microdamage model can be determined and its parameter is 0.0555 N g^{-1} of body weight maximum load, frequency of 4 Hz, 10 000 circles per time and three times per week for 2 weeks.

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Effects of Cadmium on Bone Biomechanical Properties in Male Rats

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Objective: To investigate the effects of cadmium (Cd) on biomechanical properties of femur and lumbar spine in male rats.

Methods: Twenty-four 8-week-old Sprague–Dawley male rats were randomly divided into four groups, which were given $CdCl_2$ by subcutaneous injection at the doses of 0 (sodium chloride), 0.1, 0.5 and 1.5 mg kg^{-1} body weight, respectively. At the 12th week, blood, lumbar spine and femur were collected for cadmium assay, bone mineral density (BMD) measurement and biomechanical test.

Results: Cd in blood and bone of rats treated with Cd were significantly higher that the control, $P<0.05$. BMD of rats exposed to 0.5 mg Cd/kg and 1.5 mg Cd/kg were significantly lower than the control. Biomechanical properties of femur and lumbar spine in rats treated with Cd were decreased compared with the control, especially for biomechanical properties of femur in 1.5 mg Cd/kg group and biomechanical properties of lumbar spine in 0.5 and 1.5 mg Cd/kg groups, $P<0.05$.

Conclusion: Cd exposure could reduce bone biomechanical properties and the reduction of biomechanics of lumbar spine was more sensitive than femur.

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The Influence of Osteoporosis on Wear Particles Mediated Prosthesis Osteolysis

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Objective: To study the influence of osteoporosis (OP) on wear particles mediated prosthesis osteolysis *in vivo.*

Methods: OP model was established by bilateral ovariectomy (OVX) of 6 months SD female rats. The 5-mg titanium particles

were implanted onto the calvaria of OP rats, as compared with calvaria from normal control (NC) rats. Calvaria were harvested after 14 days. Skulls were analyzed by histomorphometry to measure the area of calvarial sagittal suture osteolysis and stained with tartrate-specific alkaline phosphatase (TRACP) to count the number of osteoclasts.
Results: Compared with NC and SHAM groups, the bone mineral density (BMD) and bone histomorphometry index of OVX group were significantly reduced ($P<0.05$). Although titanium particles induced obvious calvarial sagittal suture osteolysis and osteoclastogenesis in both groups as compared with control rats, the more significant change was found in NC rats treated with 5-mg titanium particles around the calvaria (NC+TI). However, the area of calvarial sagittal suture osteolysis were 0.262±0.009, 0.130±0.013, 0.307±0.013 and 0.178±0.011 mm^2 in OP+TIOP+PBSNC+TI and NC+PBS groups, respectively.
Conclusions: The reduction of osteolytic response suggests that OP may have a protective role against particle-induced bone resorption.

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WNT/β-Catenin Signaling Involved in the Osteogenesis of BMSCs Induced Drynaria Total Flavonoids

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Objective: To study the expression of wnt/β-catenin signaling factor mRNA during drynaria total flavonoids on osteogenic differentiation of bone marrow mesenchymal stem cells (BMSCs).
Methods: Isolate BMSCs from SD rats by whole bone marrow culture method, and purify them by passage. Intervene P3 BMSCs with 100 $\mu g\,ml^{-1}$ concentration drynaria total flavonoids. After 21 days, observation was made and stained mineralized nodules. After 7, 14, 21 and 28 days of intervention, ALP activity was detected. PCR was carried out to detect wnt/β-catenin signaling pathway related factors β-catenin, LEF1 and CyclineD mRNA expression.
Results: On each time points after intervention, ALP activity was compared in cell supernatant between two groups, drynaria total flavonoids group is higher than blank control group (7 days: 11.097±0.087 vs 1.613±0.137; 14 days: 24.620±0.339 vs 1.635±0.047; 21 days: 18.407±0.058 vs 1.529±0.043; 28 days: 14.905±0.141 vs 1.519±0.039; $P<0.01$ in all time points). On the 21 day, mineralized nodules are stained with Alizarin red, drynaria total flavonoids group formed mineralized nodules, while blank control group stains negative. On the 14th day, β-catenin mRNA expression in drynaria total flavonoids is higher than blank control group (0.3570±0.0626 vs 0.1740±0.0134, $P<0.05$). On the 7th day, LEF1, Cycline D mRNA levels in drynaria total flavonoids are higher than blank control group, LEF1 0.0611±0.0002 vs 0.0345±0.0131; CyclineD 0.1510±0.0255 vs 0.0718±0.0294, $P<0.05$, in all comparisons).
Conclusion: Drynaria total flavonoids can induce BMSCs to differentiate into osteoblasts through wnt/β-catenin signaling pathway.

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The Effects of Glucose on Bone Marrow Stromal Cells Differentiation into Osteoblastic Lineages

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Objective: To observe the effects of glucose on the expression of alkaline phosphatase (ALP), bone morphogenetic protein-2 (BMP-2) and transforming growth factor-beta I (TGF-βI) for rat bone marrow stromal cells (BMSCs) differentiation into osteoblasts, and to investigate the mechanism of the impact on the bone metabolism from them.
Methods: Bone marrow stromal cells were achieved from long bone marrow of rats under aseptic condition. After purification, passage and amplification by using the whole bone marrow adherence method, the BMSCs were interfered into osteoblasts with different doses of glucose concentration (5.5 and 25.0 $mmol\,l^{-1}$) in the presence of the osteogenic medium. The rate of mineralization was examined by staining of mineralized nodules with Alizarin red S, and expression of ALP, BMP-2 and TGF-β1 was examined by enzyme linked immunosorbent assay (ELISA) after 21 days of culture.
Results: Compared with 5.5 $mmol\,l^{-1}$ glucose classic group, the rate of mineralization was reduced significantly in 25.0 $mmol\,l^{-1}$ glucose classic group (45.3±0.7 vs 68.3±0.8%, $P<0.05$). There were significant reduction observed in the activity of ALP (0.350±0.020 vs 0.563±0.043), BMP-2 (590±27 vs 744±41 $\mu g\,l^{-1}$) and TGF-β1 (875±40 vs 1,188±52 $\mu g\,l^{-1}$; $P<0.05$ in all comparisons).
Conclusions: High concentration glucose inhibits the differentiation of BMSCs into osteoblasts, which provide a potential mechanisms of diabetes-induced osteoporosis.

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Simulated Microgravity Using Random Positioning Machine Inhibits Osteoblast Differentiation and Mineralization

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Aims: The bone loss induced by microgravity is partly due to the decrease of mature osteoblasts. To get further understand of the mechanism, we used the random positioning machine (RPM) to simulate microgravity and investigated the acute effect and long-term effect of simulated microgravity on the differentiation and mineralization of osteoblasts, respectively.
Methods: A RPM was used to simulate microgravity. For the acute effect study, confluent 2T3 preosteoblasts were cultured with osteogenic medium (a-MEM containing 10% FBS and supplemented with 100 $\mu g\,ml^{-1}$ ascorbic acid and 5 mM b-glycerophosphate) for 7 days in 1 g gravity and then were set as two groups: 1 g (G) and simulated microgravity (SM). After 24 h, cells were collected for alkaline phosphatase (ALP) activity assay, quantitative real-time RT-PCR and western blot, respectively. For the long-term effect study, confluent MC3T3-

E1 cells cultured with osteogenic medium were set as two groups: G and SM. Cells were treated for 14 days and then were subjected for Alizarin Red S staining.
Results: In present study, we found that 24 h acute exposure of SM by RPM significantly reduced the ALP activity of differentiating 2T3 cells, and decreased the mRNA expression of osteogenic genes including runt-related gene 2 (runx2), osterix, osteocalcin and type I collagen. Moreover, the phosphorylated extracellular signal-regulated kinase (Erk) was increased. The long-term effect study also showed that the osteoblast mineralization was inhibited after 14 days treatment of SM.
Conclusions: The results show that the acute treatment of SM inhibits the differentiation of differentiating 2T3 cells by reducing the expression of osteogenic genes and reveal that the Erk pathway may have a negative role in this process. The long-term effect study shows that SM retards the process of MC3T3-E1 osteoblast mineralization.

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Fibronectin is Involved in Gravity Sensing of Osteoblast-Like Cell

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Aims: Extracellular matrix–integrin–cytoskeleton system is normally considered as gravity sensor of cell, but the role of extracellular matrix protein in gravity sensing is unknown. Fibronectin (FN), an abundant ECM protein, has crucial roles in cell adhesion, migration, growth and differentiation. In previous work, we found that fibronectin gene expression in MG-63 cells under simulated weightlessness condition was increased significantly compared with other conditions, and this result was verified by microarray test.
Methods: To investigate the alteration of FN on the protein level under diverse gravitational condition, osteoblast-like cell line MG-63 was cultured under diamagnetic levitation and random position machine (RPM) conditions. Cellular FN protein expression (cFN) was detected by western blot, and soluble FN was detected by ELISA. To prove whether the interference of the fibronectin's interaction with its cell surface receptor, integrin, can affect the expression of fibronectin, RGD-peptide and integrin antibody were respectively added in the MG-63 cell culture for 24 h, then soluble FN was detected.
Results: There is no significant difference between cFN in samples from diverse gravitational environments formed by superconducting magnet with large gradient. However, samples from the weightlessness (μg) always had more soluble FN in 24 h. Interestingly, hyper gravity group (2G) showed lower soluble FN than other groups. Under RPM environment, MG-63 cells secreted more FN in clinostat than that in normal culture in every sample time. And more soluble fibronectin was detected in MG-63 cells medium after co-cultured with RGD peptide and integrin antibody.
Conclusions: These results hint that changes of fibronectin in altered gravity may be involved in the interference of the interaction between fibronectin and integrin.

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Simulated Weightlessness Affects Morphology, Cytoskeleton in Osteocytes and Its Mechanism

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Aims: Bone remodeling is a dynamic process that requires the coordinated interaction of osteocytes, osteoblasts and osteoclasts, collaborating in basic multicellular units. Recently, osteocytes are believed to be the mechanosensors of bone, responding to mechanical stresses, but it is unclear exactly how osteocytes influence neighboring bone cells in weightlessness. Diamagnetic levitation technology is a novel approach for simulated weightlessness and has only recently been applied in biological research. Here we report the finding of bioeffects of large gradient high-magnetic field (LG-HMF) on the activity, morphology and actin filaments and actin-binding proteins, including vinculin, paxillin, talin and MACF1 (microtubule actin crosslinking factor) in osteocyte-like cell line MLO-Y4.
Methods: Osteocytes MLO-Y4 were cultured in MEM medium supplemented with 5% FBS under LG-HMF for 24 or 48 h. The effects of LG-HMF on morphology, cytoskeleton architecture, apoptosis and actin-binding proteins expression were investigated.
Results: The results showed that LG-HMF produced by superconducting magnet had no acute lethal effects on osteocyte-like cells. Compared to control, diamagnetic levitation (μg) obviously affected osteocytes morphology, nucleus size, actin architecture and actin-binding proteins distribution and expression. However, the effects of 1-g and 2-g apparent gravity on osteocytes were not significant.
Conclusions: The study indicates that bone cells are sensitive to altered gravity, and actin-binding proteins (vinculin, paxillin, talin, h2-calponin and MACF1) may be involved in bone cells mechanosensation. The diamagnetic levitation may be a novel ground-based space gravity simulator and can be used for biological experiment at cellular level.

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Icaritin Regulates the Differentiation of MSCs Derived from Normal and Steroids-Associated Osteonecrosis (SAON) Rabbits

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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 differentiation potential of MSCs derived from both normal and SAON rabbits.

Methods: SAON model in rabbit was established following a standard protocol. Bone marrow MSCs were aspirated from the proximal femur of normal and SAON rabbit. 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 expressions.

Results: Differentiation assay showed that the osteogenic differentiation potential declined, while adipogenic differentiation ability elevated in MSCs derived from SAON rabbit. Icaritin enhanced osteogenic differentiation of MSCs from normal and SAON rabbits in a dose-dependent manner. Icaritin upregulated Cola1, BMP2, Runx2 and osteocalcin mRNA expressions during osteogenic differentiation of MSCs, both 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 and downregulated C/EBP-β, PPAR-γ mRNA expression. PPAR-γ and aP2 proteins expression increased in SAON rabbit, while inhibited by Icaritin in both normal and SAON rabbits. The proliferation ability of MSCs derived from SAON rabbit declined, and Icaritin had no effect on its proliferation derived from either normal or SAON rabbits. Icaritin had no effect on the expression of VEGF in MSCs derived from SAON rabbits.

Conclusion: Imbalance between osteogenic and adipogenic differentiation was found in MSCs derived from SAON rabbits; Icaritin could enhance osteogenic differentiation of the MSCs in normal rabbits and partly rescue osteogenic differentiation in SAON rabbits.

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Desmethylicaritin Inhibits Adipogenesis Via Wnt/b-Catenin Signaling Pathway in 3T3-L1 Cells

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Aims: Epimedium-derived flavonoids (EFs) were able to inhibit adipogenesis. Recently, we found that desmethylicaritin was one of the metabolites of EF in serum. In this study, we investigated the inhibitory effects of desmethylicaritin on adipogenesis and the underlying mechanism.

Methods: After reaching confluence, 3T3-L1 cells were induced with differentiation medium (designated as day 0). Desmethylicaritin was added to the medium over the full course of differentiation. Oil Red O staining was used to investigate the effects of desmethylicaritin on adipogenesis at day 8. Real-time PCR was performed to quantify the mRNA expression of CCAAT/enhancer binding protein delta (Cebpd) and beta (Cebpb) at 1, 3 and 8 h, CCAAT/enhancer binding protein alpha (Cebpa), peroxisome proliferator-activated receptor gamma (Pparg), adipocyte lipid-binding protein (Fabp4) and lipoprotein lipase (Lpl), Wnt10b and β-catenin at days 2, 4, 6 and 8. Immunofluorescence was performed to observe the nuclear translocation of β-catenin at day 2. All quantitative data were presented as means±s.d. of three experiments. One-way analysis of variance followed by Tukey *post hoc* test was used to assess statistical significance at $P<0.05$ using SPSS 17.0 statistics software.

Results: Different concentrations of desmethylicaritin in 0.1, 1 and 10 μM all significantly decreased the lipid droplets of 3T3-L1 cells when compared with adipocyte control ($P<0.05$ for all), suggesting that desmethylicaritin could reduce the adipogenesis in 3T3-L1 cells. Desmethylicaritin did not affect Cebpd and Cebpb mRNA expression as well as the mitotic clonal expansion in the early phase of adipogenesis. In contrast, 10 μM of desmethylicaritin significantly decreased the mRNA expression of the following adipogenic transcription factors, Cebpa and Pparg, and adipocyte-specific genes, Fabp4 and Lpl, as compared with that in the adipogenic-induced cells without desmethylicaritin treatment ($P<0.05$ for all at days 4, 6 and 8). Further, desmethylicaritin upregulated the mRNA expression of Wnt10b decreased by adipogenic induction, but not affected the mRNA expression of β-catenin during the adipogenesis process. However, immunofluorescence results showed that desmethylicaritin increased nuclear translocation of β-catenin at day 2.

Conclusions: The present results demonstrated that desmethylicaritin inhibited adipogenesis by downregulating the expression of Cebpa and Pparg, which might be regulated via Wnt/β-catenin pathway.

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Dosing Effect of Extracorporeal Shockwave on Delayed Tendon-Bone Insertion Healing

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Objectives: To investigate whether low dose of extracorporeal shock wave (ESW) treatment would be as effective as high dose of ESW for treatment of delayed tendon-bone insertion (TBI) healing.

Method: Partial patellectomy with shielding was performed on 96 female New Zealand White Rabbits according to our established protocol. The rabbits were divided into three different groups (Control), low dose (LD-ESW; 0.06 mJ mm^{-2}, 4 Hz, 1500 impulses), and high dose (HD-ESW; 0.43 mJ mm^{-2}, 4 Hz, 1500 impulses) and two different time points (8 and 12 weeks after partial patellectomy). ESW was applied 2 weeks after the removal of shielding, which was placed between the tendon and bone after partial patellectomy, at week 4. Calcein green and xylenol orange were injected 2 weeks and 1 week before euthanasia, respectively. New bone area and volume were measured using anteroposterior radiographs and micro-

CT, respectively. Decalcified and undecalcified histological sections were assessed for fibrocartilage regeneration and for new bone forming rate, respectively. The tensile strength of the TBI healing interface was evaluated mechanically. One-way analysis of variance with Bonferroni *post hoc* test was used to detect differences between different groups. Significance level was set at $P<0.05$.

Results: Radiographic assessments showed that the new bone area was significantly larger in both LD-ESW group (62.1%, P=0.024 at week 8 and 66.1%, P=0.003 at week 12) and HD-ESW group (75.4%, P=0.015 at week 8 and 61.7%, P=0.003 at week 12) than the control at the same time point. The new bone volume in LD-ESW and HD-ESW groups was 94.9% ($P>0.05$) and 135% (P=0.034) larger than that of the Control group at week 12, respectively. Histological assessments showed the enhanced TBI healing quality with regeneration of fibrocartilage zone between the tendon and bone healing interface in the ESW-treated groups. Sequential fluorescence-labeled sections showed that new bone formation rate in both LD-ESW and HD-ESW groups was significantly higher than that of the control at week 12. The failure load of both the LD-ESW and HD-ESW groups showed 30.9% (P=0.044) and 37.7% (P=0.038) higher than that of the control group at week 12, respectively. However, there was no significant difference between the LD-ESW group and HD-ESW group at both weeks 8 and 12 for all assessments.

Conclusions: The effect of low-dose ESW was similar to the high-dose ESW in promotion of the healing in delayed TBI injuries with respect to the radiological, micro-architectural, histological and mechanical assessments.

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Bioactive PLGA/TCP Scaffolds Incorporating Phytoestrogenic Molecule Icaritin Developed for Bone Defect Repair

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Objective: The strategy presented in this study was to incorporate BMP-2 or phytomolecule icaritin (ICT) into a composite scaffold for potential enhancement of bone regeneration. We fabricated novel bioactive scaffolds, namely PLGA/TCP/BMP-2 and PLGA/TCP/ICT with three dosages of ICT (L, M and H). The hypothesis was that the original bioactivities of BMP-2 or icaritin within scaffolds could be maintained, which would serve as a local delivery system for enhancement of bone regeneration.

Methods: Scaffolds were fabricated at –28 °C by a low-temperature rapid-prototyping machine using our established protocol. The scaffolds were divided into following groups: P/T (A), P/T/LICT (B), P/T/MICT (C), P/T/HICT (D), P/T/BMP-2 (E). Based on our previous *in vitro* results with increasing ALP activity and calcium deposition of BMSCs onto scaffolds of groups B, C, D compared with that in groups A and E, we established an ulna bone defect model in rabbits to investigate bone repair enhancement by evaluation of X-ray, XtremeCT and histology at weeks 2, 4 and 8 post-surgery. ANOVA was used for statistical analysis.

Results: The fabricated scaffolds were porous with 65±5.46% porosity and 467±20.34 mm in pore diameter. At weeks 2, 4 and 8, the radiographic results and X-ray scores showed the more bone formation in the pores of scaffolds and on the surface of the implants as well as along the adjacent host bone in groups B, C, D compared with that of groups A and E. XtremeCT showed better bone formation, bone density and relative bone volume in the defect region in groups B, C, D. Histological analysis also confirmed CT results.

Discussion and Conclusion: The available results demonstrated that PLGA/TCP/ICT could significantly enhance osteogenic differentiation, as ICT was able to keep its bioactivity during fabrication procedure. However, PLGA/TCP/BMP-2 did not show initially expected osteogenic effect that was explained; the preparation and fabrication processes might have denatured the BMP-2 that was added into PLGA/TCP paste. However, its mechanisms on either enhanced osteogenesis and/or angiogenesis remained for delineation in future studies. PLGA/TCP/ICT as an innovative composite scaffold possesses osteogenic differentiation potential confirmed *in vitro* or *in vivo* studies. This may form a good foundation for potential clinical validations to use this bioactive composite scaffold for enhancing bone defect repair.

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Released Hydrogen Gas During Degradation of Mg Based Metal Alloy and its Influence on Host Tissue In Vivo

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Objectives: The degradable magnesium (Mg) became an alternative absorbable implant material for bone screws and plates in orthopedics, except polymer implant, which may avoid the second surgery for implant removal. Alloying elements plus an amorphous single-phase structure would significantly improve the corrosion characteristics of magnesium since the fast corrosion of pure magnesium. Strontium (Sr), which has no toxicity, can successfully improve the corrosion resistance and Sr is also well known of its osteoinduction. In this study, Mg-Sr alloy cylinder was prepared for implantation in mice and the hydrogen release *in vivo* were evaluated as well as the influence of the degradation of Mg alloy on the surrounding tissues.

Methods: A hole of 0.7 mm in diameter was drilled in femur along the axial of shaft from mice distal femur, and the cylinders of Mg-Sr with 5 mm in length were implanted into the hole and the hole without metal alloy worked as control. Micro-CT examination was performed every week and totally for 8 weeks after implantation. The volume of hydrogen released from Mg alloy during degradation was also evaluated using micro-CT. The degradation and mechanical properties (finite element analysis) of Mg alloy and the changes of bone around the implants were evaluated by using micro-CT and histology.
Result: The Mg-Sr alloy lost original integrity with implantation over time and then was disintegrated completely at 8 weeks after implantation. The mechanical strength of Mg alloy also decreased from week 0 to week 8 by using finite element analysis. The bone volume and the thickness around the alloys increased, higher than those in control group at 8 weeks after operation ($P<0.05$). The histology analysis showed more newly formed bone in Mg alloy group and the mineral deposit rate was higher than that in the control group ($P<0.05$). No bubbles were found in bone marrow cavity and around muscle from week 0 to week 8 in the control group, but much of them were found in Mg-Sr group. The volume of hydrogen gas increased at week 1 and week 2 and then decreased after 2 weeks in Mg alloy groups. No inflammation and osteonecrosis were found at the bone and muscle around the degraded Mg alloy and hydrogen gas. There were also no changes found in the liver and kidney of the mice.
Conclusions: Mg-Sr alloy could degrade *in vivo* and stimulate the new bone formation around the implant. The released hydrogen gas did not have adverse influence on the bone growth and other organs. These findings suggested their potential application in orthopedics, such as absorbable screw or plate for non-weight bearing skeletal sites.

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PLGA/TCP/Icaritin Scaffolds Enhance Angiogenesis in Bone Defect Healing

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Objective: Treatment of large bone defect in routine orthopedic clinics requires scaffold materials, especially desirable with composite material combined with therapeutic and bioactive agents for achieving better treatment outcome. The strategy in this study was to develop such a bioactive biodegradable composite bone scaffold incorporating a phytomolecule icaritin (ICT) for bone regeneration. To investigate angiogenesis potentials of PLGA/TCP composite scaffolds incorporating ICT in bone defect repair process, the fabricated bioactive scaffolds, namely PLGA/TCP, PLGA/TCP/ICT with three dose of ICT (L, M and H) were applied to treat bone defect and analyze angiogenesis. The hypothesis was that ICT incorporating composite scaffolds possess angiogenesis enhancement ability in bone healing that would be a possible mechanism of bone regeneration.
Methods: Scaffolds were fabricated in Tsinghua University using established protocol. The scaffolds were divided into P/T (control), P/T/LICT, P/T/MICT and P/T/HICT. We established an ulna bone defect model in rabbits to investigate the new vessels ingrowth by microfil perfusion technique followed by micro-CT-based micro-angiography at weeks 2, 4 and 8 post-surgery that can reconstruct 3D images and quantitatively measure newly formed vessels volume in pores of implanted scaffolds. In addition, local blood perfusion function in implanted scaffolds in defect area was detected by magnetic resonance imaging (MRI). ANOVA was used for statistical analysis.
Results: The fabricated scaffolds were porous with 60–65% porosity and 450–500 mm in pore diameter. At weeks 2, 4 and 8, micro-angiography results showed increasing new vessels forming in the defects over the healing time, and as lateral comparison more newly formed vessels growing in the pores of ICT incorporating scaffolds compared with that of control scaffold. We also observed that at week 4 new vessels ingrowth peaked in bone healing process and middle dose of ICT scaffolds showed the most new vessels formation that matched our bone formation enhancement results. MRI results showed the better local blood function in middle dose of ICT incorporating scaffolds in defects than that of the control scaffold.
Conclusion: The results demonstrated that PLGA/TCP/ICT could significantly enhance angiogenesis in bone healing process. However, its mechanisms on enhanced angiogenesis would be investigated in future studies. PLGA/TCP/ICT as an innovative composite scaffold possesses angiogenesis potential, that is, the accompaniment of osteogenesis. This may form a good foundation for potential clinical validations to use this bioactive composite scaffold for enhancing bone defect repair.

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Changes in Mechanical Properties of Mg-Sr, Mg-Sr-Ca and Mg-Sr-Zn During Degradation *In Vitro* and *In Vivo*

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Objectives: Alloying elements plus an amorphous single-phase structure would significantly improve the corrosion characteristics of magnesium since the fast corrosion of pure magnesium. Strontium (Sr), calcium (Ca) and zinc (Zn), which have no toxicity, can successfully improve the corrosion resis-

tance and the Sr is also well known of its osteoinduction. While mechanical support of implant is very important for load-bearing application during bone healing after bone fracture fixation, the changes in mechanical properties of magnesium alloy during degradation shall be made known before further preclinical validations. In this study, Mg-Sr, Mg-Sr-Ca and Mg-Sr-Zn alloy were prepared and their changes in mechanical properties *in vitro* and *in vivo* were evaluated, as well as the influence of the degradation of Mg alloy on the surrounding bony tissue.

Methods: *In vitro* study: the alloys were immersed into cell culture medium (pH 7.35) for 2 weeks. The mechanical properties of these alloys were evaluated using three points bending at 0, 1 and 2 weeks. *In vivo* study: under general anesthesia a hole of 0.7 mm in diameter was drilled in femur along the axial of shaft from mice distal femur, and the cylinders of Mg-Sr, Mg-Sr-Ca and Mg-Sr-Zn with 5 mm in length were implanted into the hole, and the hole without metal alloy was used as the control. Micro-CT examination was performed every week up to week 8 after implantation. The degradation and mechanical properties of Mg alloy and the changes of bone around the implants were evaluated.

Results: During degradation *in vitro*, the pH value of the medium increased gradually from 0 week to 2 weeks and the end pH value was about 10 in all three groups. Two weeks after immersion into the medium, the bending strength and elastic modulus of alloy (Mg-Sr: 264±25 MPa, 89±1 GPa; Mg-Sr-Ca: 308±53 MPa, 102±5 GPa; Mg-Sr-Zn: 337±10 MPa, 107±9 GPa) decreased when compared with those Mg-Sr (332±19 MPa, 109±2 GPa), Mg-Sr-Ca (442±5 MPa, 179±14 GPa) and Mg-Sr-Zn (439±19 MPa, 145±15 GPa) before degradation, respectively ($P<0.05$). With implantation over time, the Mg-Sr, Mg-Sr-Ca and Mg-Sr-Zn alloy lost their original integrity and the debris from the outer layer of metal materials dispersed into the medullary cavity, but the metal alloy did not disintegrate completely. The finite element analysis (FEA) of micro-CT showed that the stiffness and apparent modulus of three kinds of Mg alloys decreased from 0 week to 8 weeks ($P<0.05$). The bone volume and the thickness around the alloys increased and was higher than those in the control group at 8 weeks after operation in three groups ($P<0.05$). The mechanical strength of femora in three groups did not change significantly during the *in vivo* degradation of Mg alloys.

Conclusions: Mg-Sr, Mg-Sr-Ca and Mg-Sr-Zn alloys could degrade *in vivo* and *in vitro* and their mechanical strength decreased accordingly. Even if the mechanical strength of these Mg alloys decreased, it was also higher than that of the normal bone. The degraded elements of Mg alloys could also stimulate the new bone formation around the implant and did not decrease the mechanical strength of bone.

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Dual Effect of Puerarin on Promoting Osteogenesis and Inhibiting Adipogenesis

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Aims: Puerarin is an isoflavones extracted from the root of Pueraria Labata (Willd) Ohwi and recently found its potential effects on prevention of osteoporosis. In this study, we investigated potential dual effects of puerarin on osteogenesis and adipogenesis *in vitro*.

Methods: The cytotoxicity of puerarin (0.1, 1, 10, 20 μM) on the osteoblastic-like MC3T3-E1 and 3T3-L1 preadipocytes was investigated by CCK-8 kit. MC3T3-E1 was used to evaluate the osteogenic effects of puerarin. Alkaline phosphotase (ALP) and Alizarin Red S staining were performed on the three dosages of puerarin (0.1, 1, 10 μM). Oil Red O staining in 3T3-L1 cells were used to demonstrate the effects of puerarin (10, 20 μM) on adipogenesis. The real-time PCR was used to detect the mRNA expressions of the adipocyte-related genes, such as CCAAT/enhancer binding protein a (Cebpa), peroxisome proliferator-activated receptor g (Pparg), adipocyte lipid-binding protein (Fabp4) and lipoprotein lipase (LPL). Furthermore, immunofluorescence was used to observe the nuclear translocation of β-catenin, a key mediator of Wnt signaling pathway. All quantitative data were presented as means±s.d. of three experiments.

Results: Puerarin had no cytotoxicity effect on the selected dosages on both MC3T3-E and 3T3-L1 cell lines at 48 h after treatment. Data showed that puerarin (10 μM) caused a significant increase in ALP activity at 6 days osteogenic induction, and also in the mineral nodules in 8 days induction in osteoblasts. Those suggested that puerarin could promote osteogenesis. On other hand, puerarin decreased the adipocytes in a dose-dependent manner, as well as downregulated the mRNA expression of adipogenic transcription factors Cebpa, Pparg, and adipocyte-specific genes Fabp4 and LPL. These results suggested that puerarin could inhibit adipogenesis. In addition, immunofluorescence labeling revealed that in undifferentiated 3T3-L1 cells, β-catenin was distributed in the nuclei, and translocated into the cytoplasm when these cells differentiated into adipocytes. Importantly, treatment of these cells with puerarin restored the nuclear localization of β-catenin. Nuclear translocation of β-catenin has a key role on activating of Wnt signaling, which promotes osteogenesis and inhibits adipogenesis.

Conclusions: Puerarin can promote the osteogenesis and inhibit adipogenesis *in vitro*. The underlying mechanism might be through Wnt/β-catenin signaling pathway.

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A Rapid-Prototyped Composite Scaffold material Incorporating an Osteopromotive Molecule can Promote the Healing of Steroid-Associated Osteonecrotic Lesion in Emus

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Aims: As there is no satisfactory treatment for effective bone defect repair in surgical tunnel after removing dead bone in

steroid-associated osteonecrotic (SAON) lesions up to now, this research is set to investigate the following two specific aims:
1. To evaluate the efficacy of PLGA/TCP/Icaritin composite scaffold material on bone defect repair in the bipedal emu model with SAON lesions.
2. To examine the degradation property of the newly developed degradable PLGA/TCP/Icaritin scaffold.
Methods: All the 15 adult male emus (30 hips) were injected steroid to induce osteonecrosis for 12 weeks at the femur head and dead bone was removed from surgical tunnel with 6 mm diameter. All the emus were divided into three groups: control group without any scaffold treatment (*n*=10); PLGA/TCP group with implantation of PLGA/TCP scaffold (*n*=10) and PLGA/TCP/Icaritin group with implantation of PLGA/TCP/Icartin group (*n*=10). All the emus were scarified after 12 weeks post-surgery. Decalcified section of the surgical tunnel was performed. Histology analysis of H&E staining and immunohistochemistry staining of VEGF protein signal was carried out to investigate the new bone formation, scaffold degradation and angiogenesis by measuring and comparing the area ratio as morphology qualification.
Results: In H&E-stained decalcified sections of the surgical tunnel, new-bone-like tissues are found. The area ratio of new-bone-like tissue is much higher in the PLGA/TCP group and PLGA/TCP/Icaritin group compared the control group, in which the new-bone-like tissue can be hardly found. Moreover, the difference of the area of new-bone-like tissue is obvious in PLGA/TCP/Icaritin group compared with the PLGA/TCP group. However, the ratio of aligned collagen fiber in these new-bone-like tissue is lower. These results may indicate that the majority of new bone formation was still in the early stage without been mineralized. The low percentage of mineralized tissue in the surgical tunnel is also illustrated in the CT analysis. High expression of signaling protein VEGF is shown in the surgical tunnel of PLGA/TCP/Icaritin group, which indicates the enhanced angiogenesis in the scaffold. Morphometric analysis shows that the degradation rate of scaffold is higher in PLGA/TCP/Icaritin group than that of the PLGA/TCP group.
Conclusion: In conclusion, both PLGA/TCP and PLGA/TCP/Icaritin scaffold can promote osteogenesis and angiogenesis in bone defect healing in bone defect after core-decompression in steroid-associated osteonecrosis. PLGA/TCP/Icaritin scaffold provides better treatment efficacy and higher degradation rate than PLGA/TCP scaffold after implantation *in vivo*.

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Polymorphisms in the GALNT3 Gene are Associated with Bone Mineral Density and Fracture Risk in Chinese Postmenopausal Women

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Aims: Osteoporosis has a strong genetic component, but its exact genetic background is still poorly understood. We studied whether GALNT3, a gene associated with hyperphosphatemic familial tumoral calcinosis, is an osteoporosis-risk gene by examining association between its polymorphisms and bone mineral density (BMD), osteoporotic fractures and vertebral fractures in Chinese postmenopausal women.
Methods: Overall, 1607 postmenopausal women were randomly selected from the Peking Vertebral Fracture (PK-VF) study in Beijing. BMD of lumber spine, femoral neck and total hip were measured by dual-energy X-ray absorptiometry. Vertebral fracture phenotypes were ascertained by vertebral X-ray reading. Osteoporotic fracture phenotypes were obtained from questionnaire. Single-nucleotide polymorphisms (SNPs) of GALNT3 were determined by TaqMan allelic discrimination assay. We used multiple statistic methods to test the association between SNP genotypes and phenotypes of osteoporosis.
Results: 1. Polymorphisms of rs13429321, rs6710518 and rs1863196 were significantly associated with femoral neck BMD (*P*-value was 0.005, 0.003 and 0.020, respectively) as well as total hip BMD (*P*-value was 0.002, 0.000 and 0.002, respectively). 2. Individuals carrying genotype TT of rs13429321, TT of rs6710518 or GG of rs1863196 had higher risk of osteopenia and osteoporosis in femoral neck. 3. Osteoporotic fractures were associated with rs6721582 and rs4667836. Homozygous and heterozygous of minor allele increased the İncidence of fractures by 1.444 (95% CI 1.023–2.037, *P*=0.037) and 1.560 (95% CI 1.124–2.163, *P*=0.008) fold, respectively.
Conclusions: In our study, polymorphisms of rs13429321, rs6710518 and rs1863196 were significantly associated with femoral neck BMD and total hip BMD. Polymorphisms of rs13429321, rs6721582 and rs4667492 were associated with fracture risk. This is the first report about the association between these allelic variants and the phenotypes of postmenopausal osteoporosis in Chinese population. GALNT3 may have a role in the genetic susceptibility to osteoporosis and fracture among Chinese postmenopausal women.

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Menatetrenone vs Alfacalcidol 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 with lower bone mass in older women and men. The aim of this 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 alfacalcidol (Haier) 0.25 μg, twice per day for 1 year. Patients also received elemental calcium 500 mg. BMD and biochemical markers including serum total 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 lumbar spine and trochanter, respectively, in the menatetrenone group (M group) compared with baseline BMD ($P<0.001$). In the alfacalcidol group (A group), BMD was also increased by 2.2 and 1.8% at lumbar spine and trochanter, respectively ($P<0.001$). There were no changes in femoral neck BMD in either group. No differences in changes were found between the two groups after 6 and 12 months of treatment ($P>0.05$). After 12 months treatment, OC and ucOC were decreased by 38.7 and 82.3% in 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 declines of serum OC and ucOC were 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 alfacalcidol.
Conclusions: With 1-year treatment by menatetrenone, the BMD of lumbar spine and hip increased in postmenopausal osteoporotic patients. Menatetrenone treatment has similar effects on BMD to alfacalcidol. 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.

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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 of metabolism of bone and lipid are involved in the complex pathogenesis of nontraumatic osteonecrosis of femoral head (ONFH). A previous study confirmed that adiponectin predominantly exhibited significant anti-inflammatory and anti-atherosclerotic effects. These indirectly prevented obstruction of blood vessels that leads to some 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), respectively. 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^{-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 correlate 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 assessing the potential risk of nontraumatic ONFH.

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Analysis of 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: Under the permission by the Ethics Committee of the Hospital and the patients, the femoral heads of patients were collected 2 h after the surgery of total hip replacement. Fifty-two 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 the femoral heads along the plane perpendicular to stress direction of bone under physiological conditions. Morphological and mechanical analysis was performed on surgical samples, such as DXA scan, three-dimensional microstructure scan with the 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 statistical lower in the field of volumetric bone mineral density, tissue bone mineral density and bone mineral content measured by DXA. 2. *Microstructure*: Compared with other group, the osteoporosis group was statistically lower in bone mineral content, area, bone mineral density, bone trabecular number and bone volume fraction ($P<0.05$), while the osteoporosis group was statistically increased 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 lower in volumetric ash content and percentage of ash content ($P<0.05$), while it was increased in the field of percentage of organic content ($P<0.05$); but it was not statistically significant in volumetric organic content. 4. *Biomechanics*: Osteoporosis group was significantly lower in elastic stress, elastic modulus and maximum stress ($P<0.05$), while it was significantly increased in change of height after fatigue test ($P<0.05$). 5. *Bone metabolic marker*: Osteoporosis group was significantly lower 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 maintain the ability of fracture resistance mainly through enhancing the anisotropy of trabeculae, while osteonecrosis group achieve primary bone strength by increasing 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 osteoporosis group is more obvious than the other.

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Clinical Value of Serum Total-P1NP, β-CTX and 25(OH)D3 Determination in Fragility Hip Fractures of the Osteoporotic Elderly

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Objective: To investigate the clinical value of serum total procollagen type 1 aminoterminal propeptide (Total-P1NP), cross-linked C-terminal telopeptide of type I collagen (β-CTX) and 25(OH)D3 measurement in the setting of fragility hip fractures of the osteoporotic elderly.

Methods: Serum levels of Total-P1NP, β-CTX and 25(OH)D3 were measured in 68-year-old osteoporotic people diagnosed with fragility hip fracture, and in 68 age- and gender-matched osteoporotic controls without fragility hip fracture; bone mineral density (BMD) was detected by dual X-ray absorptiometry. SPSS16.0 software was used to analyze the data.

Results: (1) Serum levels of Total-P1NP and β-CTX in the fragility hip fracture group were higher than in the control group; serum levels of 25(OH)D3 in the fragility hip fracture group were lower than the control group ($P<0.05$); BMD of the lumbar spine and total hip did not differ significantly between the two groups. (2) Bivariate correlation analysis suggested that in the fragility hip fracture group, the serum 25(OH)D3 level was positively related and serum Total-P1NP and β-CTX levels were negatively related to BMD of the lumbar spine and total hip ($P<0.05$); in the control group, 25(OH)D3 was not related to BMD of the lumbar spine or total hip. Serum Total-P1NP and β-CTX levels were negatively related to BMD of the total hip ($P<0.05$), but not related to BMD of the lumbar spine.

Conclusion: In osteoporotic elderly people with similar BMD levels, high serum level of Total-P1NP and β-CTX, and low serum level of 25(OH)D3 might independently indicate high risk of fragility hip fracture. Detection of the three markers may be helpful in detecting and treating people with high risk of fragility hip fracture.

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The Association Between Metabolic Syndrome and Bone Mineral Density: A Meta-Analysis

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Aims: Previous research demonstrates uncertainty about the effect of metabolic syndrome on bone. We performed a meta-analysis to investigate the association of metabolic syndrome with bone mineral density of the spine and femoral neck.

Methods: In this meta-analysis, searches of Medline, Embase, Cochrane Library, Chinese biological medical database (CBM) and China national knowledge infrastructure (CNKI) were undertaken to identify studies in humans of the association between metabolic syndrome and bone mineral density. Random effects model was used for this meta-analysis. The results of our research were reported using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.

Results: A total of 11 studies (including an outlier study) with 13 122 subjects were included in our research. We detected a significant overall association of metabolic syndrome with increased bone mineral density of the spine (WMD=0.027, 95% CI (0.011, 0.042)) and no significant overall association of metabolic syndrome with bone mineral density of the femoral neck (WMD=0.008, 95% CI (–0.011, 0.026)). Subgroup analyses indicated significant association between metabolic syndrome and increased bone mineral density of the spine in subjects whose bone mineral density was measured by a DXA scanner manufactured by Hologic, subjects diagnosed by IDF criteria and subjects diagnosed by NCEP-ATPIII criteria. We also noted significant association between metabolic syndrome and increased bone mineral density of the femoral neck in Caucasian subjects, subjects whose bone mineral density was measured by a DXA scanner manufactured by Hologic, and subjects diagnosed by NCEP-ATPIII criteria.

Conclusions: Our meta-analysis suggests that metabolic syndrome has no clear influence on bone mineral density, or its influence may be beneficial.

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Association of the Type 2 Diabetes Mellitus and Osteoporosis in Postmenopausal Women with the BMP-4 Gene Polymorphism

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Objective: The aim of this study was to explore the distribution of polymorphism bone morphogenetic proteins-4 (BMP-4)

gene and the association of BMP-4 gene with glucose metabolism, bone remodeling and bone mineral density (BMD) in postmenopausal women with type 2 diabetes mellitus (T2DM) and osteoporosis (OP) in Shanghai region.

Methods: A total of 485 unrelated postmenopausal women of Han nationality in Shanghai region were recruited and divided into four groups. Group A (OP group, 120 cases), group B (T2DM group, 108 cases), group C (T2DM with OP group, 130 cases) and group D (healthy control group, 127 cases). The bone mineral density of lumbar spine L2–4 and femoral neck, bone alkaline phosphatase (BALP), tartrate-resistant acidphosphatase-5b (TRAP-5b), glycosylated hemoglobin A1c (HbA1c) and fasting plasma glucose (FPG) were measured.

Results: In the groups A and C for the spine L2–4 and femoral neck, BMD, the HH genotype was significantly higher than hh genotype ($P<0.001$); the Hh genotype was significantly higher than the hh genotype ($P<0.001$). There was no significant difference in the L2–4 and femoral neck BMD between the BMP-4 HH genotype and Hh genotype in the groups A and C (*P*-value=0.118 and 0.800).

Conclusion: BMP-4 genotype might be susceptibility gene for postmenopausal OP in Shanghai region, and h allele is associated with low bone mass. The H allele is associated with high bone mass relation. BMP-4 genotype is not associated with glycosylated hemoglobin.

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Bone Metabolism of Patients with Rheumatoid Arthritis and Clinical Characteristics of Rheumatoid Arthritis-Associated Osteoporosis

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Objective: Rheumatoid arthritis (RA) is a common systemic disease that manifests as symmetric polyarthritis. Recently, many researchers have observed that osteoporosis (OP) is common in RA patients. The purpose of this study was to assess incidence, clinical characteristics and bone metabolism of RA-associated OP.

Methods: Sixty RA inpatient Chinese patients (44 women and 16 men) diagnosed with RA were analyzed. Bone mineral density (BMD) of RA patients was measured through dual-energy X-ray absorptiometry (DXA). Serum levels of osteoprotegerin (OPG) and bone gla protein (BGP) were determined, and compared with normal controls. RA patients were divided into OP group and non-OP group according to BMD. Characteristics of RA-OP in clinical and laboratory data were analyzed. Age, sex distribution, duration of disease, clinical and laboratory parameters were compared between the patients with and without OP.

Results: The incidence of normal bone mass, low BMD and OP were 18.33, 25 and 56.67%, respectively, in 60 RA patients. The incidence of OP in female RA patients was higher than that in male (29/44 vs 5/16, $P<0.05$) and so was in the postmenopausal than in the premenopausal (28/36 vs 1/8, $P<0.01$). The levels of BGP of patients with RA were significantly higher than normal controls (9.73±3.90 vs 4.83±2.02 $\mu g\,l^{-1}$, $P<0.01$), while the levels of serum OPG was significantly lower (71.08±33.47 vs 106.19±41.08 $ng\,l^{-1}$, $P<0.01$). The RA-OP group was older (57.8±13.9 vs 48.0±12.6 years, $P<0.01$), had a lower mean level of serum Ca (2.19±0.14 vs 2.30±0.17 $mmol\,l^{-1}$, $P<0.01$) and higher levels of serum ALKP (89.7±31.4 vs 73.5±22.7 $U\,l^{-1}$, $P<0.05$) and OPG (78.73±33.77 vs 61.06±30.90 $ng\,l^{-1}$, $P<0.05$) compared with non-OP group. No statistical significance was obtained in disease duration, the levels of RF, BGP, ESR, CRP or P ($P>0.05$).

Conclusions: There was a high incidence of OP in RA patients, especially in the postmenopausal women or elderly cases. A high bone remodeling rate was observed in RA patients, which was more pronounced in those who also had OP.

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Trabecular Bone Score In Normal Ukrainian Women Of Different Age

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Aim: This study is evaluating Trabecular Bone Score (TBS) in normal women of different age.

Materials and methods: We have examined 176 normal women aged 40–79 years (mean age –53.4±0.6 years; mean height –163.5±0.5 cm; mean weight –80.4±1.1 kg). The patients were divided into the following age-dependent groups: 40–49 years (*n*=53), 50–59 years (*n*=89), 60–69 years (*n*=17), 70–79 years (*n*=17). TBS (L1–L4), total body, lumbar spine, femoral neck bone mineral density (BMD), lean and fat masses were measured by DXA using a densitometer Prodigy, GE.

Results: We have determined the significant decrease of TBS (L1–L4) in women with age (40–49 years—1.334±0.016 mm^{-1}; 50–59 years—1.289±0.013 mm^{-1}; 60–69 years—1.194±0.034 mm^{-1}; 70–79 years—1.205±0.050 mm^{-1}; F=6.56; $P=0.0003$). BMD of spine significantly increased with age (BMD of spine: 40–49 years—1.126±0.015 $g\,cm^{-2}$; 50–59 years—1.234±0.013 $g\,cm^{-2}$; 60–69 years—1.343±0.053 $g\,cm^{-2}$; 70–79 years—1.348±0.100 $g\,cm^{-2}$; F=4.04; $P=0.008$). BMD of femoral neck did not show significant differences. The significant correlation was observed between TBS (L1–L4) and age, fat and lean masses:

-TBS=1.64–0.007×age; $r=-0.34$; $t=4.41$; $P=0.00002$.
-TBS=1.47–0.000005×total fat (g); $r=-0.37$; $t=4.86$; $P=0.000003$.
-TBS=1.90–0.00001×lean mass (g); $r=-0.59$; $t=8.98$; $P<0.000$.

We did not find significant correlation between TBS and BMD of spine and femoral neck:

-TBS=1.36–0.05×BMD of spine; $r=-0.05$; $t=0.66$; $P=0.5$.
-TBS=1.53–0.22×BMD of femoral neck; $r=-0.16$; $t=1.94$; $P=0.05$.

Conclusion: The significant correlation between TBS and lean mass indicates that bone quality can be associated with muscular system. TBS was significantly decreased with age. TBS is an independent parameter that has potential diagnostic value without bone mineral density.

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The Therapeutic Response to Alendronate with Osteoporosis and N740N Polymorphism of Low Density Lipoprotein Receptor Related Protein 5 (LRP5) Gene

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Objective: To investigate whether the change of bone mineral density (BMD) after alendronate 1-year treatment in postmenopausal patients with osteoporosis associates with the N740N polymorphism of *low-density lipoprotein receptor-related protein 5* (*LRP5*) gene and determine the correlation between genotypes and the therapeutic effect.
Methods: Overall, 67 postmenopausal osteoporosis patients were recruited with the average age of 64.2±7.7 years. Every patient took oral alendronate (Forsamax) 70 mg weekly and caltrate 600 mg daily for 12 months. Pre- and post-treatment bone mass density were measured at lumbar spine 2–4 and left hip sites. PCR-RELP was performed for the N740N polymorphism of *low-density lipoprotein receptor-related protein 5* (*LRP5*) gene.
Results: One-year therapy was accomplished in 63 patients. Compared with the baseline BMD, the post-treatment BMD was increased significantly at all sites. The BMD of lumber spine 2–4 was increased (4.05±3.39%), while the BMD of femoral neck was increased (1.05±2.63%), the BMD of torch was increased (2.00±2.89%), the BMD of inter-torch was increased (2.17±2.62%) and the BMD of total hip was increased (1.79±2.29%) ($P<0.01$). In all 63 patients, the frequencies of CC and CT genotypes were 65.1 and 34.9%. TT genotype was not found. The distribution followed the Hardy–Weinberg equilibrium. Of the post-treatment and the percentage of BMD, CT genotype changed above CC genotype at the torch and total sites were found ($P<0.05$). However, the two genotypes were not significantly associated with the percentage of BMD at the L1–4, the neck and the inter sites.
Conclusion: The BMD of lumbar spine was increased more significantly than the BMD of hip sites in postmenopausal osteoporosis Chinese women after 1-year alendronate therapy. Also there is correlation between the therapeutic response and the N740N polymorphism of *low-density lipoprotein receptor-related protein 5* (*LRP5*) gene. Lager studies are needed to confirm these results.

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X-Ray Absorptiometry Indexes for Women in Postmenopausal Period with Osteoporotic Fractures

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Aim: To estimate the structural and functional conditions of bone in women in postmenopausal period with osteoporotic fractures, compare the results to referent data for a Ukrainian population and to compare the results of X-ray absorptiometry to the fracture risk rate, assessed by FRAX for women in postmenopausal period with osteoporotic fractures.
Objective: Thirty-nine women in postmenopausal period aged 50–89 years with forearm (n=18) and proximal hip (n=21) fractures, who were treated in the Traumatology Department #1 of Lviv City Clinical Hospital of Ambulance were included. They were divided into four categories by age (50–59 (n=13); 60–69 (n=12); 70–79 (n=9); 80–89 (n=5)).
Methods: Nordin Index was measured with the 'Osteolog' workstation, developed in the Institute of Gerontology AMS Ukraine under the direction of professor VV Povoroznyuk. Fracture risks were estimated using FRAX.
Results: We found lower cortical indexes for women in postmenopausal period with osteoporotic fractures for 50–59 (Common IN=0.41), 60–69 (Common IN=0.40), 70–79 (Common IN=0.36), 80–89 (Common IN=0.33) age groups in comparison to referent data for Ukrainian population. Also we found lower cortical indexes for women in postmenopausal period with higher risk of osteoporotic fracture, assessed by FRAX, independent of age.
Conclusion: Thus, low cortical indexes, measured with the 'Osteolog' workstation, are reliable predictors of high fracture risk. There is a significant correlation between low cortical indexes and high fracture risk, assessed by FRAX.

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Proximal Humeral Internal Locking System (PHILOS) for Osteoporotic Displaced Proximal Humeral Fractures

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Objective: Fractures of the proximal humerus only account for 4–5% of all fractures with 80% requiring no surgical intervention. Mainly elderly patients with poor bone quality were affected and most fractures belong to osteoporotic fractures. However, the management of the other 20% fractures remains controversial. Locking plate technology offers mechanical advantages for treating unstable fractures in osteoporotic bone.
Methods: This study included a series of 36 patients who had proximal humeral internal locking system (PHILOS) plate fixation for proximal humeral fractures. The mean follow-up was 15 months. The mean age of patients was 68.5 years. The fractures were classified according to the Neer system.
Results: There were no cases of infection, nerve injury, nonunion, osteonecrosis or hardware failure. No loosening of implants and cutout occurred. Subacromial impingement was observed in two patients and received implant removal. The mean Constant score was 73.8. The mean SF-36 scores for physical and social functions were 68.7 and 88.0, respectively. These results indicate that the PHILOS plate offers stable fixation and good functional outcomes in an elderly population group with low rates of complications. In the management of osteoporotic displaced proximal humeral fractures, it may have specific advantages for early mobilization.
Conclusion: We recommend this as an effective option, although careful technique and patient selection are crucial.

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Effect of Strontium Ranelate On Vertebral Pain Syndrome and Functional Abilities in Postmenopausal Women with Systemic Osteoporosis

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Aim: To evaluate the effect of strontium ranelate in the treatment of systemic osteoporosis in postmenopausal women.
Materials and methods: There were examined 894 postmenopausal women with systemic osteoporosis (average age 59.97±10.57 years, average height 161.82±7.09 cm, average weight 71.32±13.44 kg). Evaluation of pain syndrome and level of physical activity was carried out with visual analog scale (VAS). Examination was performed before the onset of treatment and after a 4-, 8- and 12-month treatment course. Strontium ranelate (Bivalos, 'Servier') was taken in a dose of one 2 g sachet as a suspension in water once a day and one tablet of Calcemin-advance (Calcium—500 mg, Vit. D—400 IU) two times a day during 12 months.
Results: The patients had the risk factors of osteoporosis: 28% of patients had osteoporotic fractures in their anamnesis; 17%—hip fractures in mother or father of patients, 12%—smoking, 8%—alcohol abuse, 27% of patients had taken corticosteroid tablets for more than 3 months. We observed a reliable decrease of vertebral pain syndrome (after treatment –2.97±0.77, after 4 months –2.24±0.85, after 8 months –1.61±0.94; after 12 months –1.24±1.04; $P<0.00001$) and increase of functional abilities of patients (after treatment –1.50±0.67, after 4 months –2.08±0.52, after 8 months –2.67±0.53; after 12 months –2.88±0.63; $P<0.00001$).
Conclusion: It has been demonstrated that strontium ranelate treatment significantly decreases pronounced vertebral pain syndrome and improves functional abilities of patients in the postmenopausal women.

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Postmenopausal Osteoporosis at Women with Diabetes Type 2

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Introduction: Diabetes mellitus (DM) is a known risk factor of fractures. The aim of study is to research dynamic of parameters of mineral metabolism after 1 year of therapy with zoledronic acid in women with postmenopausal osteoporosis and diabetes type 2.
Materials and methods: Twelve women (age 65±3.2 years) in postmenopausal period (menopause –46.1±1.5 years) with verified diabetes type 2 (diabetes present from 1 to 12 years; all patients had standard oral therapy with an anti-diabetes drug) and postmenopausal osteoporosis (DEXA—*T*-score less than –2.5) were included. Bone mineral density (DEXA), serum C-telopeptide crosslink, calcium and alkaline phosphates were monitored for 1 year.
Results: A total of 16.7% of patients had hyperemia (37.2–38.1) (resolved during 1–2 days). Bone mineral density at DEXA *T*-score was 21.5±2.86% ($P=0.05$), lumbar spine BMD was 16.7%, L2—18.4%, L3—25%, femoral neck—24.6%, Wards—16.9%, Troch—27.3% and total hip—23.35% ($P=0.05$). Calcium and alkaline phosphates remained in the normal range. Overall, 58% of women had a pain syndrome (in spine or in the hip), and after 2 months of the therapy 14.3%, 14.3% after 4 months, 57.1% after 6 months and 14.3% after 1 year of treatment. No patient had hypoglycemia. None suffered either nonvertebral or vertebral fracture.
Conclusions: Zolendronic acid may reduce risk of nonvertebral and vertebral fracture in patients with osteoporosis and diabetes type 2.

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Analysis on Therapeutic Compliance and its Influential Factors Among Postmenopausal Women on Osteoporosis Medications

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Objective: To explore the therapeutic compliance of the postmenopausal women with osteoporosis and factors influencing this.
Methods: Clinical data of 200 postmenopausal women who were diagnosed with osteoporosis by dual energy absorptiometry in the Prevention and Treatment Center of Osteoporosis, Huadong Hospital, Fudan University, from March 2010 to March 2011 were collected. Follow-up was carried out at 3, 6 and 12 months to determine the status of the drug use. Patients who stopped treatment were asked for the reasons for discontinuation. All patients were investigated using a questionnaire. Logistic regression was used to evaluate the relationships between influential factors and discontinuation.
Results: Discontinuation rates and reasons in patients at different time periods of treatment were not the same. The cumulative discontinuation rate was 35% in 12 months. The influential factors of discontinuation included education, family history of osteoporosis, symptoms, using more than five kinds of drugs, relief, convenience, side effect and cost.
Conclusion: The therapeutic compliance of the postmenopausal women with osteoporosis is poor. We can improve compliance by enhancing patient's education about osteoporosis and treatment, strengthening the management of medication in elderly patients, taking appropriate measures in different time periods of treatment and identifying the patients at high risk of withdrawal.

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Wrong Evidence-Based Medicine, Osteoporosis and Targeted Breast Cancer Therapies

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Background: Researchers looked at women aged 50–79 years taking a combination of conjugated equine estrogens 0.625 mg and medroxyprogesterone acetate 2.5 mg. This confirmed the long held assumption that HRT prevents osteoporotic fractures. HRT was widely prescribed to women to relieve

the menopausal symptoms. Researchers found that women who took the hormones had an increased risk of developing breast cancer.
Aim: Searching for new answers in the link between breast cancer, osteoporosis and targeted breast cancer drugs.
Method: We have performed a bibliography review in a worldwide basis and from our own experience.
Results: Breast cancer risk is strongly related to age, with 81% of cases occurring in women aged 50 years and over. Breast cancer is the most common cancer in women. The lifetime risk of developing breast cancer is one in eight for women. Risk factors associated with the disease could be viruses, environmental factors or others acting on breast cell. Women in developed countries are at increased risk of breast cancer compared with women from less developed countries. A large part of this variation can be explained by the fact that women in developed countries have fewer children on average and a limited duration of breastfeeding, it is said. However, in reality reproductive factors that influence breast cancer risk do not explain it. Female breast cancer incidence rates vary considerably, with the highest rates in the Europe and the lowest rates in Africa and Asia. Breast cancer is one of the few cancers where incidence rates are higher for more affluent women, and there is a clear trend of decreasing rates from least to most deprived groups. Postmenopausal osteoporosis usually affects women over the age of 60 years. The leading cause of osteoporosis is a lack of estrogens in women and opposite drugs. Osteoporosis, affects one in two women, is now three times more common than breast cancer. Bisphosphonates may contribute to fewer breast cancers. The cell cycle consists of four phases. DNA and RNA viruses have been shown to be able to cause cancer and are referred to as carcinogens.
Conclusions: The natural lack of estrogen does not decrease breast cancer incidence. Targeted breast cancer therapies are to be studied. Environmental factors could have any role, but viruses can attack cells in different phases and that could explain the different breast cancer types.

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The Recent Security Observation to the Zoledronic Sodium

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Objective: To understand zoledronic sodium phosphate the recent adverse after medication.
Methods: November 2009 to February 2012, 156 cases of osteoporosis patients were treated with zoledronic sodium phosphate, clinical manifestations were observed in patients within 1 month of medication, focusing on pain, fever and other adverse events, over the same period 107 cases with spinal fractures treated by Vertebroplasty but not zoledronic sodium phosphate were compared.
Result: This group of patients were all followed up. The results showed that the incidence of pain was 17.3%, incidence of fever was 12.8% in zoledronic sodium group; the incidence of pain was 1.87%, incidence of fever was 0.93% in the control group, the difference was significant ($P<0.001$). On the other hand, the following were observed in zoledronic sodium phosphate group: one case of skin allergies; one case of myocardial ischemia and angina pectoris; one case of sicker patient with multiple myeloma; one case of pneumonia and died of respiratory failure at last.
Conclusion: The administration of zoledronic sodium in the osteoporosis could cause increased pain, fever and other side effects, and may even cause skin allergies, angina, and exacerbations of multiple myeloma, pneumonia, respiratory failure and other adverse reactions, recommendations to medication under the closely supervision at that day, and to strengthen the first 2 weeks of follow-up to patient after medication.

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Absence of Germline LEMD3 Mutations in Sporadic Patients with Osteopoikilosis

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Aims: Osteopoikilosis is a rare asymptomatic bone dysplasia of unknown etiology. It is usually found incidentally on radiological examinations. Melorheostosis is characterized by a 'flowing' hyperostosis of the cortex of human bones, often coexist with osteopoikilosis. Mutations in the *LEMD3* gene were detected in familal osteopoikilosis with or without melorheostosis patients, but were rarely found in sporadic patients. We investigated LEMD3 in two sporadic patients with osteopoikilosis.
Methods: A 26-year-old female and a 21-year-old male with osteopoikilosis and melorheostosis were collected from outpatient department of our hospital. They were all diagnosed accidentally by X-ray examination. Radiographs showed multiple, small, bone islands within the hands, feet, tibias, femurs, vertebral bones, and bilateral long bones had melorheostosis. DNA was extracted from the peripheral blood. LEMD3 exons and adjacent mRNA splice sites were amplified by polymerase chain reaction (PCR) and sequenced in both directions.
Results: No germline LEMD3 mutations were found in these two patients.
Conclusions: The present study supports the general conclusion that. LEMD3 germiline mutations were rarely detected in sporadic osteopoikilosis. The genetic or epigenetic influences that are responsible for the development of osteopoikilosis and melorheostosis requires further investigation.

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Clinical Observation on Changes of Serum Bone Turnover Indicators of Adult Female

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Objective: To analyze the changes of serum Procollagen Type I Intact N-terminal Propeptide (PINP), β-Crosslaps, N-MID

Osteocalcin (N-MID) and parathyroid hormone (PTH) in 40–80 women, as well as their correlations with bone mineral density (BMD).
Methods: The levels of PINP, β-Crosslaps, N-MID and PTH were tested by electrochemiluminescence immunoassay; BMD was measured by American GE company's Lunar Prodigy dual-energy X ray (DEXA) bone density meter.
Results: 1. Serum PINP, β-Crosslaps and N-MID of the 50–59 group increased significantly ($P<0.05$), followed by slight falls, and PTH increased significantly in the 70–80 group ($P<0.05$). 2. PINP, β-Crosslaps and N-MID are negatively correlated with BMD of all parts (r=–0.134/–0.256, $P<0.05$), and PTH is negatively correlated with BMD of FN and Troch (r=–0.138/–0.201, $P<0.05$). Compared with normal group, PINP and N-MID of both low bone mass (except Ward's) and osteoporosis groups were significantly higher ($P<0.05$); β-Crosslaps of both low bone mass and osteoporosis groups were significantly higher ($P<0.05$); at L1–L4 and FN, PTH of the low bone mass group was significantly higher ($P<0.05$), at FN, Wards and Troch, PTH of osteoporosis group was all significantly increased ($P<0.05$).
Conclusion: High bone turnover is an important cause of bone loss in women. PINP, β-Crosslaps, N-MID and PTH are sensitive and specific bone biochemical markers that are indicators of the bone turnover change with age in women, monitoring these indicators will help to prevent and treat osteoporosis earlier.

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Age-related Changes of Bone Biochemical Markers and Their Relationships to Mineral Density in Men

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Objective: To study the age-related changes of serum Procollagen I N-terminal Propeptide (PINP), β-Crosslaps and N-MID Osteocalcin (N-MID) in men, as well as their correlations with bone mineral density (BMD).
Methods: The levels of serum PINP, β-Crosslaps and N-MID were tested by electrochemiluminescence immunoassay; BMD of lumbar spine 1–4, femoral neck, wards, troch and femur were measured by American GE company's Lunar Prodigy dual-energy X-ray (DEXA) bone density meter.
Results: PINP, β-crosslaps and N-MID were negatively correlated with age, as serum PINP, β-Crosslaps and N-MID were relatively high and stable at the age of 30–49 years, then PINP decreased gradually with aging to a nadir level at the age group of 60–69 years, while β-Crosslaps and N-MID declined to the lowest point in the age group of 80 years. After adjustment for age, height, weight and BMI, serum PINP and β-Crosslaps were negatively correlated with BMD of multiple skeletal sites; N-MID was inversely associated with BMD of multiple skeletal sites, except L1–4. Analysis of variance showed, while men over 70 years divided into groups according to BMD, the PINP, β-Crosslaps and N-MID of osteoporosis group were significantly higher than that of normal bone mass group; β-Crosslaps and N-MID of osteoporosis group are significantly higher than that of the low bone mass group.
Conclusion: PINP, β-Crosslaps and N-MID are sensitive and specific bone biochemical markers, which are indicators of the bone turnover in men. Monitoring these markers help the prevention and early treatment of osteoporosis. In men over 70 years, the bone biochemical markers of low bone mass and osteoporosis group were significantly increased compared with that of the normal bone mass group, suggesting that bone turnover of these male osteoporosis patients was high, and the high bone turnover caused quick lose of bone mass.

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Minimal Invasive PFNA to Treat Osteoporotic Intertrochanteric Fracture of the Old

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Object: We evaluated the usage of proximal femoral nail antirotation blade (PFNA) for the treatment of osteoporotic intertrochanteric fracture of the old.
Methods: From January 2007 to August 2011, we applied PFNA to 69–year-old patients (age >65 years old, averaged 76.2 years) with intertrochanteric fracture caused by falling. Bone density was assessed by Single index and ultrasonic bone densitometry of calcanei, and fracture type was assessed by AO classification. ASA sore, operation duration incision length, hospital stay, amount of surgical bleeding, aggravation of the original disease was also recorded. Postoperative hip function was evaluated by Harris hip score.
Result: According to single and ultrasonic bone densitometry of calcanei, osteoporosis rate was 95.4%. AO classification: 14 cases of A1, 31 cases of A2, 20 cases of A3. ASA sore was II to III, and the average surgical time 52 min, incision length 3–5 cm, surgical bleeding 250 ml, average hospital stay 15 days and all patients received no aggravation of the original disease. All patients were followed up with Harris hip score, good and excellence rate 87.7% (excellence in 33 patients, good in 24, 7 in fair and bad in 1).
Conclusions: PFNA has the advantage of short surgical duration, small incision, less surgical bleeding, no aggravation of original disease and fewer trauma, strong fixation, antirotation. Thus, it is believed that PFNA is an ideal internal fixation material for all type of intertrochanteric fracture of the femur with osteoporosis.

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Analysis of Clinical Effect of Radius Distal Comminuted Fracture Treated with Locking Compression Plate Combined with Bone Autograft in Elderly Patients

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Objective: To investigate the clinical therapeutic effect of radius distal comminuted fracture treated with locking compression plate (LCP) combined with bone autograft in elderly patients with osteoporosis.

Methods: From August 2009 to August 2011, 19 patients with radius distal comminuted fractures were treated with LCP combined with bone autograft. Male 12 patients and female 7 patients, and the average age was 58.5 years, all the patients had osteoporosis. In all patients, falling was in 15 cases, traffic accident was 3 cases and heavy bruise was in 1 case. Palm side of the wrist surgical approach was used, the fracture was fixed with LCP, and the bone defect was filled with autologous iliac bone graft. The patients were followed up after operation. Clinical follow-up included wrist function and radiographic films to investigate the bone union.
Results: All patients were followed up, the average follow-up was 1.5 years. The results of wrist function were evaluated, excellence was 10 cases, fair was 6 cases, good was 2 cases and poor was 1 case. All the fractures were union, and there was no infection and malunion. Palm inclination and ulnar deviation degrees of the wrist after surgery improved significantly compared with preoperative degrees.
Conclusion: It is an effective method to fix radius distal comminuted fracture with locking compression plate (LCP) combined with bone autograft. Patients can perform early functional exercise after operation, which especially suite to elderly patients with osteoporosis.

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The Study on Preparation of AMS Target Samples with Rats Femur

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Objective: Calcium is one of the important elements that form human bone (the main form of calcium is $Ca_{10}(PO_4)_6(OH)_2$). It participates in and regulates many life processes. Osteoporosis is the most common disease of calcium deficiency. It is a serious threat to human health, especially for old people, but recent research shows that organisms (especially osteoporosis organisms) take in too much calcium, possibly causing some other diseases. Thus, a clear calcium metabolism regulation mechanism is meaningful to the study of osteoporosis. Calcium isotope tracer technology is an effective method to study the biological effects of calcium. As the best tracer of all calcium isotopes, ^{41}Ca can only be tested through accelerator mass spectrometry (AMS). It is very important to pay attention to how to prepare target samples efficiently to meet the demand of AMS, as well as how to reduce the content of interference nuclide in samples.
Materials and methods: The materials that AMS uses to test ^{41}Ca are hydrogenated calcium and fluoride calcium. Hydrogenated calcium has strong beam intensity and transmission efficiency, which can effectively reduce the interference of 41K. However, the process of producing hydrogenated calcium is very complex, and the sample is hard to store, costing too much to prepare and measure large biological samples. This experiment takes a rat's femur (containing ^{41}Ca) to make fluoride calcium samples, which meet AMS measurement standards. Through some physical and chemical processes such as ion exchange, secondary fluorination, we deal with the rat's femur samples and explore a preparation process that uses fluoride lead as a conductive medium. Finally, we press the mixture, which uses CaF_2/PbF_2 as 1/4 to targets, and save them in the dry argon.
Results and Conclusion: The result shows that the scheme of the preparation of the sample reduces the interference of 41K effectively and improves the beam intensity. After the examination, 41K in the samples decreased to lower than 1×10^{-6}, and further measurements by CIAE–AMS system found that the scheme was able to improve the beam intensity of CaF3– to be three times greater than before. In addition, the interference of 41K in the lead was strongly reduced and the measured sensitivity of ^{41}Ca could achieve about 10=14 in the samples. It is able to completely meet the demand of radioactive tracer with ^{41}Ca for osteoporosis patients.

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A Novel Compound Mutation of CYP27B1 in a Chinese Family with Vitamin D-Dependent Rickets Type 1

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Objective: Mutations in the *CYP27B1* gene, which encodes vitamin D 1α-hydroxylase, are the genetic basis for vitamin D-dependent rickets type 1 (VDDR-I, MIM 264700). The aim of this study was to investigate the CYP27B1 mutation and its clinical manifestations.
Materials and methods: The proband was 5-year-old male child who had frequently hands, legs, perioral twitching and growth retardation since the age of 12 months. Laboratory data showed hypocalcemia, low urine calcium, high serum alkaline phosphatase and elevated PTH. His parents were not of consanguineous marriage background, also no similar history was found in the family. The height of his father and mother were 176 and 168 cm, respectively. The entire coding region of the *CYP27B1* gene was sequenced in the proband, the other family members and 200 healthy donors.
Results: The proband was found with compound heterozygotes: two missense mutations in *CYP27B1* gene located in the 7 and 8 exons. The proband parents carried the above heterozygous mutation, but one of his brother and 200 healthy controls did not carry the above mutation.
Conclusions: According to the database HUMD mutation, the above-mentioned compound heterozygous mutations was a novel mutation, not yet reported in the literature, which also provided a new basis for further research and clinical diagnostics in VDDR I.

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The A242T Mutation in the Low-Density Lipoprotein Receptor-Related Protein 5 Gene in One Chinese Family with Osteopetrosis Autosomal Dominant Type 1

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Osteopetrosis autosomal dominant type 1 (OPTA1) (OMIM: 607634) is a type of rare inheritable bone disease. A Chinese family with two affected individuals is reported in the present study to investigate the clinical characteristics and virulence gene of this sclerosing bone disorder. Biochemical and radiographic examination, bone mineral density (BMD) and genetic analysis were performed in two patients and eight other family members. The 40-year-old proband (II-1) and her 64-year-old mother (I-2) both had chronic lumbodorsal pain, an elongated mandible and torus palatinus in the center of the hard palate. No fracture was observed in any of the family members. The skull, mandibular and pelvic X-rays presented thickened cranial plates, an enlarged sella turcica, an elongated mandible and cortical thickening of the long bones. The BMD value of the two patients was significantly higher compared with the standard age- and sex-matched adult mean reference values. Both patients had a higher serum sclerostin level, while their renal function marker, serum calcium and phosphonium, PTH and 25(OH)D levels were within the normal range. The heterozygous missense mutation p.Ala242Thr in exon 4 of the *low-density lipoprotein receptor-related protein 5* gene (*LRP5*) was detected in two patients, while the other family members and 200 healthy donors had normal wild-type genotypes. We conclude that the A242T mutation in *LRP5* results in the high bone mass phenotypes with an elongated mandible and torus palatinus in this OPTA1 family.

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Fibroblast Growth Factor 23 and Klotho are Present in the Growth Plate

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Introduction: The regulation of phosphate homeostasis is essential for mineralization and enchondral ossification. Fibroblast growth factor 23 (FGF23) and its obligatory co-receptor Klotho (KL) have a key role in this process by influencing both renal phosphate reabsorption and vitamin D metabolism. In disease, excessive action of FGF23 leads to hypophosphatemic rickets, while its deficiency causes tumoral calcinosis. Although osteocytes and osteoblasts are widely seen as the primary source of FGF23 under physiological conditions, the origin of systemic FGF23 remains controversial.
Materials and methods: Tissue samples were obtained from 4- to 6-week-old piglets. mRNA expression was quantified by real-time PCR and normalized to 18S rRNA. Immunohistochemical staining was performed for FGF23, Klotho, ColX and FGFR1. Growth plate chondrocyte sub-populations were acquired by collagenase digestion of growth plate explants and subsequent density gradient centrifugation.
Results: In this study, we investigated the expression of FGF23 and KL in porcine growth plate cartilage, adjacent tissues and parenchymal tissues. We could clearly detect both FGF23 and KL mRNA and protein in growth plate chondrocytes. FGF23 expression was mainly found in hypertrophic and resting chondrocytes. Furthermore, significant expression of both genes was observed in bone, liver and spleen.
Conclusion: Our data challenge previous expression analyses, in particular, theories of bone as the exclusive source of FGF23. Moreover, significant expression of FGF23 and KL within the growth plate and adjacent tissues implies a potential local role of FGF23 in chondrocyte differentiation and tissue mineralization.

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Ionizing Radiation Response in Preosteoblast Differentiation and Mineralization

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Objective: Bone loss can be seen in astronauts traveling in space as well as patients who receive therapeutic radiation. However, until now little is known about the molecular effects of radiation regarding bone cells, especially concerning the bone-forming osteoblasts.
Methods: To evaluate modifications concerning the differentiation and mineralization process of osteoblasts after exposure to ionizing radiation (IR), we directed OCT-1 cells to the osteogenic lineage by treatment with a combination of b-glycerophosphate, ascorbic acid and dexamethasone.
Results: The main characteristic of mature bone cells is their ability to deposit extracellular matrix that mineralize under *in vitro* culture conditions. *In vitro* mineralization was evaluated based on histochemical staining and quantification of the hydroxyapatite content of the extracellular bone matrix. Expression of mRNA encoding Runx2 and osteocalcin (OCN) was analyzed. Notably, calcium content analysis revealed that deposition of mineralized matrix after IR exposure tracks in a time-dependent manner under osteogenic conditions, whereas calcium deposition was slightly detectable after exposure to different doses of IR in standard medium. In this study, the higher radiation doses exert significant overall effects on Runx2 and OCN gene expression, suggesting that gene expression following IR treatment is affected in a dose-dependent manner. Additionally, we verified that Runx2 was suppressed within 24 h after irradiation at 2 and 4 Gy. Although further studies are required to verify the molecular mechanism, our observations strongly suggest that treatment with IR markedly alters the differentiation and mineralization process of preosteoblastic cells. New molecular insights will help to develop treatment strategies to prevent dangerous bone loss in astronauts as well as patients receiving radiation therapy.

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Trace Elements Correlation of the Long and Mix Bone

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Objectives: One of the most important functions of the osseous tissue is depositing of macro- and microelements, due to this research on the skeleton ion profile is highly important to explain the osteogenesis physiological and reparative processes. The osseous tissue comparing to blood is more stable system and rather difficult to be investigated, as the material collection should be performed without any harm on an organism, so it is under investigated yet. That is why not only the level of microelement concentration is important, but also its balance too, because some elements have incomplete or hypernormal routes of exposures. These factors determined the goal of research, which deals with the microelement compounds of tibia, femoral bone and II lumbar vertebra.
Methods: This study dealt with the analysis of osseous tissue samples of 20 five-month-old white rates. We have been analyzed the levels of Fe, Mg, Zn, Cu, Co, Mn, Ni, Pb and Cd. The experiments were performed using the setup C 115-01 with flame atomizer ('Selmi', Ukraine) and double-beam atomic absorption setup KAS 120.1 ('Selmi', Ukraine) with deuterium corrector of non-atomic absorption. The research results were checked according to generally accepted methods of analysis of variance, with the help of MX Excel software program.
Results: Analysis of the elementary compound of different bone types shows diversities between tubular and mix bones microelement compound. The lumbar vertebrae contain great deal of essential elements; it clarifies its remodeling activity and enzymes availability, which allow the process. Correlation analysis of derived data figured that osseous tissue had weak positive microelement correlation interactions, while negative interactions partly identified. The low correlation coefficient values are determined by the fact that normal osseous tissue is enough stable system.
Conclusions: Correlation analysis of derived data figured that osseous tissue had weak positive microelement correlation interactions, while negative interactions partly identified. The low correlation coefficient values are determined by the fact that normal osseous tissue is enough stable system. That is why these values can be used as standard measurements for the further microelement interaction researches of various pathology osseous tissue being.

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Altered Serum Levels of Bone Metabolism Markers in Rheumatoid Arthritis

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Background and Objective: Rheumatoid arthritis (RA) is a chronic inflammatory disease leading to joint destruction and disability. Previous studies showed that both generalized and peri-articular osteoporosis occur in RA. New biochemical markers of bone formation (that is, osteocalcin, Oc) showed contradictory results in different studies, although markers of bone resorption (that is, urinary collagen crosslinks) have shown significant increase in patients with RA. This study aimed to evaluate the serum levels of bone metabolism markers and their correlation with clinical and biological parameters that reflect the activity and severity of RA.
Methods: 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 tesed by chemiluminescence in 22 patients with active RA, as well as in 44 age- and gender-matched healthy controls. Serological and clinical parameters that reflect the activity and severity of RA, as well as radiographic joint destruction (Sharp score) were collected and correlated with bone metabolism markers.
Results: Serum CTX-I level was significantly higher in RA patients compared with age- and gender-matched healthy controls (0.59±0.37 vs 0.37±0.17, P=0.021). No significant difference was found between RA patients and healthy controls in serum PINP or N-MID.OC level. Spearman's correlation test showed serum PINP and N-MID.OC levels of RA patients correlated negatively with early morning stiffness (r=–0.589 and –0.452, P=0.006 and 0.045, respectively) and pain visual analog scales (VAS) (r=–0.444 and –0.597, P=0.039 and 0.003, respectively), but correlated positively with gripping power (r=0.748 and 0.783, P=0.005 and 0.003, respectively). Serum CTX-I level of RA patients correlated positively with rheumatoid factor level (r=0.425, P=0.049).
Conclusion: There were increased bone resorption and altered skeletal bone metabolism in RA. Biochemical markers of bone formation PINP and N-MID.OC may be a helpful biomarker for disease activity in RA.

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Low Level of Cadmium Exposure Stimulated Osteoclasts Differentiation

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Objective: Low level of cadmium exposure could result in low bone mineral densty, high risk of osteoporosis and bone fractures. However, the mechanism is still not completely clarified. It has been showed that osteoclasts may be the target cells for cadmium toxicity. In this study, we observed the effects of cadmium on osteoclast differentiation.
Methods: 1. Mature osteoclasts were isolated from long bones of newborn male and female rats as described (Chambers and Magnus, 1982). The femora, tibiae and humeri were placed in α-MEM (containing 15% FBS, Gibco) on ice until dissected free of soft tissue, curetted in room temperature media, and agitated to release bone-associated cells. The dispersed cell solution was plated into well and incubated in 5% CO_2 at 37 °C for 30 min to facilitate attachment of osteoclasts. At the third hour, different concentrations of cadmium (0.03 and 0.125 $\mu m l^{-1}$) were added for 72 h. Cadmium effects on osteoclasts and pit formation were observed. 2. RAW264.7 cells were suspended in DMEM containing 10% FBS and plated at a concentration of 2.5×10^3 cells per well into a 48-well culture plates (Corn-

ing, NY) and incubated for 24 h. Then, different concentrations of Cd (0–60 nmol l^{-1}) with or without RANKL (40 ng ml^{-1}) were added to the cultures. The medium and factors were replaced every 2 days. After 5 days' culture, osteoclasts number and mRNA expression were investigated. 3. Primary osteoblast and RAW264.7 cells were co-cultured in ratio of 1:1.5 for 5 days. Then TRACP5b in the supernatants was measured. In addition, OPG and RANKL expression was determined by RT-PCR.
Results: 1. Low level of cadmium exposure stimulated osteoclasts formation. 2. Cadmium could dose-dependently induce differentiation of osteoclasts precursor into osteoclasts in the presence of RANKL. RANK, TRAF6 and FRA may involve in those progress. 3. Cadmium could obviously stimulate RAW264.7 cells differentiation into osteoclasts and stimulated TRACP5b secretion in the presence of osteoblast. 4. Camium could obviously upregulate RANKL expression and downregulate OPG expression.
Conclusions: Low level of cadmium exposure could stimulate osteoclasts formation and differentiation; OPG/RANKL may be one of the important factors involved in cadmium effects on osteoclast.

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A Novel Homozygous Mutation in the HPGD Gene Causes Primary Hypertrophic Osteoarthropathy

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Objective: Primary hypertrophic osteoarthropathy (PHO) is a hereditary disorder featured by digital clubbing, pachyderma, hyperhidrosis and periostosis. Mutations in *HPGD* gene located on chromosome 4q34.1 have been identified in PHO patients. This gene encodes 15-hydroxyprostaglandin dehydrogenase (15-PGDH), and mutations cause an elevation of circulating PGE_2, thus the consequent manifestations in primary hypertrophic osteoarthropathy. Different mutations have been reported in families of the European and west Asian origin. We investigated the mutation in *HPGD* gene in three Chinese families.
Methods: Three previously unreported Chinese families with one or more individuals affected with typical primary hypertrophic osteoarthropathy were characterized clinically. Genomic DNA was extracted from whole blood samples, and genetic analysis was performed by direct sequencing of PCR products of *HPGD*.
Results: All three probands were young males in their second decades. The general clinical manifestations include digital clubbing, arthropathy, hyperhidrosis and hyperkeratosis. Acro-osteolysis, periosteal reaction along with cortical thickening were found in radiography. HPGD sequence analysis identified a novel homozygous mutation, c.310_311delCT (p.Leu104AlafsX3), in all the affected individuals in the three families. This mutation locates in exon 3, which alters the open reading frame from residue 104, truncating the 15-PGDH protein and causing the deletion of active site and NAD binding site, thus results in loss of enzymatic function.
Conclusion: A novel homozygous HPGD mutation, c.310_311delCT (p.Leu104AlafsX3), was identified. This mutation is a frame-shift mutation, and causes the loss of NAD binding site. This mutation appears to be unique to Chinese population.

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Large Gradient High Magnetic Field Affects Formation of Osteoclast-Like Cells

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Aims: To investigate whether gravity alteration could affect preosteoclast-like differentiation.
Methods: Preosteoclastic FLG29.1 and RAW264.7 cells were exposed to different apparent gravities (μg, 1, 2 g) in large gradient high magnetic field (LGHMF) for 3 and 4 days, respectively. Cell proliferation was detected by MTT method. Cell cycle was detected by FACS. Cell apoptosis and necrosis were assayed by staining cells with Hoechst and PI. The FLG29.1 cells were induced to form mature osteoclasts with the addition of TPA, while RAW264.7 cells were stimulated with RANKL. After cells were exposed to LGHMF with induction reagent, TRAP-positive cells and NO release were detected by TRAP staining and Griess method, respectively. Intracellular TRAP activity was measured using pNPP as the substrate. Furthermore, gene expressions of MMP9 and TRAP in FLG29.1 cells were assayed by RT-PCR.
Results: MTT detection revealed that both μg and 2 g conditions promoted cell proliferation compared to control. 1-g condition also promoted cell proliferation of RAW264.7 μg condition, increased the percentage of RAW264.7 cells at S phase and FLG29.1 cells at G1 phase. Hoechst-PI staining showed that LGHMF promoted cell apoptosis and necrosis. Exposure to LGHMF inhibited NO concentration of supernatant. Both the TRAP activity and the number of TRAP-positive cells were higher in cells of μg group than that of 2 g group. While in 1-g group, TRAP activity and the number of TRAP-positive cells were decreased significantly compared with control for FLG29.1 cells. RT-PCR revealed that both TRAP and MMP-9 showed the highest expression level in μg group.
Conclusions: These findings indicate that LGHMF could directly affect the proliferation and differentiation of preosteoclast-like cells. Reduced gravity enhanced mature osteoclast formation, while high magnetic field exerts an obvious inhibition.

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3D Architectural Developmental Patterns of Young and Adult Rats in Response to Daily Parathyroid Hormone (PTH) Administration

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Objective: We investigated the differences in 3D microarchitectural developmental patterns of young and adult rats in response to daily PTH administration by registered *in vivo* μCT scans.

Methods: Saline (Veh) or PTH (80 μg kg^{-1}) was injected daily to 1-month and 3-month-old rats for 12 days (n=3 in each age and treatment group). A 4-mm section below growth plate of the proximal tibia was scanned every 4 days by Scanco vivaCT 40 at 10.5 μm resolution for PTH groups. Scans were less frequent in vehicle group (d0 and d12), while PTH exerts a protective effect against radiation to allow multiple scans. For 3-month group, all the follow-up scans were registered to the baseline scan. For 1-month PTH group, a 2-mm bone section in the secondary spongiosa (II SP) was compared with the registered corresponding volume in the primary spongiosa (I SP) in the 4-day earlier scan.
Results: *One-month PTH group*: Rapid bone growth caused I SP translate into II SP toward diaphysis at a rate of 0.25 mm per day. There were increases of 74% in bone volume fraction (BV/TV) and 39% in trabecular number (Tb.N), and a 67% decrease in spacing (Tb.Sp) in the I SP at day 8 compared to day 0. Consistently, the corresponding volume in II SP at day 12, which was translated from I SP at day 8, caused increases of 86% in BV/TV, 46% in Tb.N and 17% in trabecular thickness (Tb.Th) than the II SP at day 4 (translated from I SP at day 0). Furthermore, we found decreases of 22–24% in Tb.N, but increases of 13–21% in Tb.Th in the II SP at days 4, 8 and 12 compared with the corresponding volume in the I SP 4 days earlier. *Three-month PTH group:* Minimal translation from I SP to II SP was observed in the adult group due to the lower growth rate. In the PTH group, BV/TV gradually increased by 9, 24, and 39% at days 4, 8 and 12 compared to day 0, primarily caused by increased Tb.Th by 7, 23 and 37% at days 4, 8 and 12 than day 0. Tb.N and Tb.Sp remained constant from day 0 to day 12. *Vehicle group:* No significant change was found for any bone volume or structure parameters in the 1-month or 3-month vehicle group.
Conclusions: We conclude that PTH directly affected I SP and the fast growth rate in young rats mediated the translation of improved bone volume and structure into II SP. In contrast, PTH increases trabecular bone mass in adult rats by thickening the existing trabeculae.

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Comprehensive Association Analysis of vitamin D Pathway Genes with Serum 25-Hydroxyvitamin D Levels Among Healthy Chinese Subjects

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Introduction: Vitamin D deficiency is a common public health problem all over the world. Recent studies indicated that genetic factors might have an important role in the determination of serum 25(OH)D levels in Caucasians and American Africans. However, genes contributing to the wide variation in serum 25(OH)D in Chinese are generally unknown.
Methods: In this study, we screened 14 important genes in vitamin D metabolic pathways using 95 single-nucleotide polymorphism (SNP) markers in a group of 3069 unrelated healthy Chinese subjects. Significant confounding factors that may affect serum 25(OH)D variations were used as covariates for association analyses. An association test for quantitative trait was performed to evaluate the association between candidate genes and serum 25(OH)D levels.
Results: Variants and/or haplotypes in *GC*, *CYP2R1* and *DHCR7/NADSYN1* have been identified as being associated with 25(OH)D levels. Participants with three or four risk alleles of the two variants (*GC*-rs2282679 and *CYP2R1*-rs10766197) were at increased risk of having 25(OH)D concentrations lower than 50 nmol l^{-1} (2.094, 1.394–3.146, P=7.4×10^{-5}) compared with those with no risk alleles. Each additional copy of risk-allele of the two variants was significantly associated with 0.061 decrease in log-25(OH)D concentrations (P=9.1×10^{-9}).
Conclusion: The results suggest that the *GC*, *CYP2R1* and *DHCR7/NADSYN1* genes might contribute to variability in the serum 25(OH)D levels in a healthy Chinese population in Shanghai. These markers could be used as tools in Mendelian randomization analyses of vitamin D, and they could potentially be drug targets in the Chinese population in Shanghai. These markers can be used as tools as Mendelian randomization analyses on vitamin D, and they provide some potential for the use as drug targets in Chinese population from Shanghai.

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The Influence of Osteoporosis on Wear Particles Mediated Prosthesis Osteolysis

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Objective: To study the influence of osteoporosis (OP) on wear particles mediated prosthesis osteolysis *in vivo*.
Methods: Forty-two female Sprague–Dawley (SD) rats aged 6 months were randomly equally divided into seven groups: the A1, A2, A3, B1, B2, B3 and C1 group. The A1, A2 and A3 groups were operated by bilateral ovariectomy, and the C1 groups were sham. After 3 months, BMD and bone histomorphometry were analyzed and compared between A1, B1 and C1 groups. The 5-mg titanium particles were implanted onto the calvaria of A2 and B2 groups, as compared with PBS onto A3 and B3 groups. Calvaria were harvested after 14 days. Skulls were analyzed by histomorphometry to measure the area of calvarial sagittal suture osteolysis.
Results: Compared with NC (B1) and SHAM (C1) group, the bone mineral density (BMD) and bone histomorphometry index of OVX (A1) group was significantly reduced (P<0.05), and the area of calvarial sagittal suture osteolysis, respectively, were 0.262±0.009, 0.130±0.013, 0.307±0.013 and 0.178±0.011 mm^2 in A2, A3, B2 and B3 groups.
Conclusions: The reduction of osteolytic response suggests that OP may have a protective role against particle-induced bone resorption.

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Effect of Hugu Capsule on BMD and Histomorphometry in Glucocorticoid-Treated Rats

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Objective: To study the effects of administration of Hugu Capsule (CH) on histomorphometry in glucocorticoid (GC)-treated rats.
Methods: Thirty 3-month-male Sprague–Dawley rats were randomized into three groups: age-matched normal control (Nrm), methylprednisolone (Met), (5.0 mg kg^{-1}, subcutaneously, per day for 5 days per week), Met plus CH orally (150 mg kg^{-1} per day). The study period was 12 weeks. DXA was evaluated in the femoral diaphysis and L5. Histomorphometry was performed in the proximal tibial metaphysis and tibial diaphysis.
Results: Met significantly decreased BMD compared with Nrm. CH significantly increased BMD compared with Met. Met markedly decreased Tb.N, %Tb.Ar, MS/BS, MAR, BFRs and increased Tb.Sp, ES/BS compared with Nrm. CH showed significantly increased Tb.N, %Tb.Ar, MS/BS, MAR, BFRs and decreased Tb.Sp, ES/BS compared with Nrm.
Conclusion: Our findings suggest CH has a greater effect according to BMD and histomorphometry analysis in preventing GC-induced osteoporosis. CH might be applicable as a bone therapeutic agent to treat secondary osteoporosis in clinic.

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Recovery of Bone Loss in Ovariectomized Rats: Effect of Synthetic Bone Mineral (SBM)

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We developed compounds described as synthetic bone mineral (SBM) that have been shown to minimize bone loss induced by mineral or estrogen deficiency in rats.
Objective: This study aimed to test the hypothesis that SBM can also recover bone loss in osteoporotic rat model.
Methods: Sham-operated (n=20) and ovariectomized, OVX (n=30) Sprague–Dawley rats were kept on low mineral diet (LMD) for 3 months. Ten rats from each group were killed and the remaining rats were divided into three groups (n=10 per group): Sham operated (GA), OVX (GB), OVX on diet supplemented with 2% SBM (GC) for additional 2 months. After killing, the bones (femur, spines) were separated, cleaned, stored in alcohol and analyzed using DEXA, microradiography, microCT and scanning electron microscopy to determine bone mineral density, bone mineral content, bone volume/total volume, porosity. Results were expressed as average±s.d. and analyzed using ANOVA.
Results: OVX on LMD experienced bone loss compared with sham-operated rats. DEXA, microradiographic (Faxitron) and microCT measurements showed that the bone loss was recovered in OVX rats that were on low mineral diet supplemented with SBM.
Conclusions: The compound, synthetic bone mineral (SBM), administered as a supplement can minimize bone loss induced by estrogen deficiency and can also recover bone loss. These results suggest that SBM may have a potential for osteoporosis therapy.

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Intervention Timing of Strontium Treatment on Estrogen Depletion-Induced Osteoporosis in Rats: Bone Microstructure and Mechanics

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Purpose: To evaluate the effect of intervention timing of Sr treatment on trabecular bone microstructure and mechanics.
Methods: Ninety female rats were randomly divided into three groups: Sham treated with vehicle (Sham), ovariectomized treated with vehicle (OVX) and ovariectomized treated with Sr compound (SrC). Each group was divided into three subgroups according to the timing of first administration of vehicle or SrC, which was at week 0 (Sham-0, OVX-0 and SrC-0), week 4 (Sham-4, OVX-4 and SrC-4) and week 8 (Sham-8, OVX-8 and SrC-8) after the operation, respectively. The treatment lasted for 12 weeks. The trabecular bone biomechanical properties, trabecular bone tissue mechanical properties, trabecular bone microstructure and bone remodeling were analyzed with mechanical testing, nanoindentation, microCT and histomorphometry, respectively. The osteoblast and osteoclast phenotypic genes were analyzed with real-time polymerase chain reaction (PCR).
Results: Early and mid-term Sr treatment (SrC-0 and SrC-4) significantly increased the ultimate load of trabecular bone compared with OVX-0 and OVX-4 groups, respectively (+16 and +19%). BV/TV was significantly higher in SrC-0 and SrC-4 groups compared with OVX-0 and OVX-4 control groups, respectively (+20 and +22%). Tb.N was significantly higher in SrC-0 and SrC-4 groups compared with OVX-0 and OVX-4 control groups, respectively (+15 and +16%). Osteoid surface (OS/BS) was significantly higher in SrC-0 and SrC-4 groups compared with the OVX-0 and OVX-4 control groups, respectively (+15 and +17%). Similarly, osteoblast surface (Ob.S/BS) was significantly higher in SrC-0 and SrC-4 groups compared with the OVX-0 and OVX-4 control group, respectively (+22 and +13%). Mineralizing surface (MS/BS) and bone formation rate (BFR/BS) were significantly higher in SrC-0 group compared with the OVX-0 group (+10 and +12%). The osteoblast-related genes (Runx2 and OC) mRNAs were significantly greater (+180 and +60%), while the osteoclast-related genes (Ctsk and TRAP) mRNAs were significantly lower (–32

and –44%) in SrC-0 group compared with the OVX-0 control group. Late Sr treatment failed to exert significant effects on any of those parameters.
Conclusions: The beneficial effect of Sr was dependent on the intervention timing in ovariectomized rats.

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Differences in Distal Radius Intracortical Porosity in Chinese and Caucasian Premenopausal Women
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Introduction: Chinese women have lower fracture rates, smaller appendicular bones with thicker cortices, thicker but fewer trabeculae. The endocortical and intracortical surface (haversian canals) are contiguous and so we speculated that reduced excavation of the (smaller) medullary canal producing the thicker cortex also results in less porosity than in Caucasians.
Methods: Distal radius images from high-resolution peripheral quantitative computed tomography (HR-pQCT, XTreme-eCT, Scanco) were processed in 23 healthy premenopausal Chinese and 59 Caucasian women (18–46 years) using Strax1.0, a non-threshold-based image analysis algorithm that segments the mineralized matrix volume and void volumes of the compact-appearing cortex, the transitional and trabecular regions. The proportion of void was quantified as the average of void spaces in each voxel.
Results: Chinese women were shorter. Porosity of the compact cortex and outer transitional zone both were lower in Chinese than Caucasians (21.2 vs 23.3%, 31.6 vs 33.6%; both $P<0.05$). The porosity of the inner transitional zone was similar by race (77.0 vs 77.9%, NS). The relatively larger cortical area within a smaller bone in Chinese was attributed to a larger compact mineralized cortex than Caucasians (25.5 vs 20.3%, $P<0.001$), not due to lower void volume.
Conclusion: Lower fracture risk in Chinese women may be partly due to less porous but thicker cortex in a smaller bone—more bone within the bone than in Caucasians.