Free Paper Session V: Basic Science

FP5.1

Combined Treatment of Vibration and β-Hydroxy β-Methylbutyrate Supplementation Reduces Adipogenesis in Muscle-derived Stem Cells and Intramuscular Fat Infiltration in Sarcopenic Mice

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Introduction: Adipose tissue metabolism plays an important role in normal muscle physiology and in the pathogenesis of intramuscular fat infiltration in sarcopenia progression. We hypothesised that low-magnitude high-frequency vibration (LMHFV) and β-hydroxy β-methylbutyrate (HMB) would slow the progress of sarcopenia by reducing fat infiltration through inhibiting adipogenesis in muscle-derived stem cells.

Methods: Senescence-accelerated mouse-P8 male mice were randomly divided into control, HMB, LMHFV, and HMB+LMHFV groups. Interventions started at age 7 months and assessed at 1, 2, and 3 months by morphological and functional tests. Muscle-derived stem cells isolated from gastrocnemius were characterised, randomised into control, LMHFV, HMB and HMB+LMHFV groups assessed by oil-red-O (ORO) staining, mRNA and protein expression. Data analysis was performed with one-way analysis of variance.

Results: The LMHFV and HMB+LMHFV groups presented lower ORO areas than the control group. Type I muscle fibre in the control group was higher than that in all treatment groups. Twitch, tetanic, and specific-tetanic forces were significantly higher in the HMB+LMHFV group than those in the control group. The specific-twitch force of both LMHFV and HMB+LMHFV groups were higher. Grip strength of HMB, LMHFV and HMB+LMHFV groups were higher. In vitro, MDSCs in the HMB, LMHFV and HMB+LMHFV groups presented lower ORO area than the control group after adipogenic induction. β-catenin mRNA in control group was lower than HMB and HMB+LMHFV groups. Dependence of β-catenin in all treatment groups was shown by si-RNA knockdown of the β-catenin.

Conclusion: The results of the present study suggest that HMB supplementation and LMHFV treatments are easy, safe and manageable interventions at the community level effective for control of sarcopenia. The results of this study support their application at the community level either alone or in combination for older people who fail the first line sarcopenia screening. Further studies are required to confirm these results in human patients.

Age-related Changes of Bone Quality and Osteocyte Activity in Male C57BL/6J Mice

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Introduction: This study aimed to examine age-related bone quality changes in C57BL/6J mice and to investigate whether these changes are associated with osteocyte activity.

Methods: Micro-computed tomography was used to measure trabecular and cortical architecture in the right femur and tibia of the mice between age 1 and 12 months. Osteocyte mRNA markers in bone tissues was determined by quantitative polymerase chain reaction.

Results: Bone mineral density and bone volume density of trabecular bone were greatest at age 1 month and declined steadily thereafter. Trabecular bone loss was characterised by decreased trabecular number, increased trabecular spacing, and decreased connectivity. In contrast, trabecular bone thickness increased to a maximum value at age 6 months and declined slowly thereafter. Cortical bone thickness increased from age 3 to 6 months and was maintained or slightly decreased thereafter. Cortical bone area of the distal femur increased from age 3 to 12 months and then declined. The cortical bone area of the midshaft cortical bone increased to a maximum value at age 6 months. Stiffness and failure load of the cortical bone peaked at age 6 months and then declined. The mRNA level of osteocyte markers such as Dmp1, E11, and SOST increased from age 3 to 6 months and declined thereafter.

Conclusions: The age-related changes in bone quality in C57BL/6J male mice were similar to those found during human ageing. Similar pattern of change was observed in osteocyte particularly in cortical bone. The C57BL/6J mice maybe an appropriate surrogate to study age-related bone loss.

New Source of Sclerostin Contributes to Delayed Healing in Challenging Bone Fractures

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Targeting Aberrantly Distributed Sclerostin-producing Fibroblasts to Enhance Rotator Cuff Repair

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Aberrant Circulating Wnt16 Expression in Adolescent Idiopathic Scoliosis

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Introduction: Low bone mass is a systemic and persistent condition in adolescent idiopathic scoliosis (AIS). Wnt16 was recently reported to be a key determinant of cortical bone thickness. We investigated whether Wnt16 affects bone remodelling in AIS, which could result in reduced cortical bone quality.

Methods: In total, 74 patients with AIS (age 13.71 ± 1.39 years) and 73 control patients (age 14.17 ± 1.02 years) were recruited. Anthropometric parameters were measured with standard stadiometry techniques, and bone quality was assessed with dual-energy X-ray absorptiometry (DXA) and high-resolution peripheral quantitative computed tomography (HR-pQCT). Multiple selective circulatory bone markers were measured with multiplex assays and Wnt16 was measured with enzyme-linked immunosorbent assay. Comparisons between groups and correlation analysis within each group were performed with SPSS.

Results: The DXA (Z-score of femoral neck areal bone mineral density) and HR-pQCT (total and cortical volumetric bone mineral density and thickness) parameters showed significantly lower bone mineral density in patients with AIS compared with control patients. Patients with AIS (Cobb’s angle of 29.00 ± 13.22°) had lower circulatory levels of Wnt16, but significantly higher levels of P1NP and osteocalcin (bone formation markers). In the control group, circulatory Wnt16 was positively and significantly correlated with circulatory levels of sclerostin and DKK-1 (Wnt signalling antagonists); this association was less apparent in patients with AIS.

Conclusion: This is the first study demonstrating an aberrant Wnt16 expression level in patients with AIS. Further validation studies with larger cohorts with different severities and in-depth mechanistic studies are warranted.
**FP5.6**

**Oral Magnesium and Vitamin C Supplementation Alleviates Steroid-associated Osteonecrosis in Rats**

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**Introduction:** Pulsed steroid treatments for infectious diseases and rheumatoid diseases often induce steroid-associated osteonecrosis (SAON) in patients. Magnesium (Mg) and vitamin C (VC) are commonly used nutrition supplements. They both have bone-anabolic and antioxidative effects.

**Methods:** In 24-week-old male Sprague-Dawley rats, SAON was induced with lipopolysaccharide and methylprednisolone. Mg (50 mg/kg/day), VC (100 mg/kg/day), or Mg+VC was orally administrated daily for 2 or 6 weeks (n=8/group/time point). Serum was collected, MICROFIL perfusion was performed for angiography, and bilateral femora and tibiae were collected for micro-computed tomography (CT) and histological examination for SAON. p<0.05 was considered significant.

**Results:** TUNEL staining showed that apoptosis of osteocytes in the Mg, VC, and Mg+VC groups was significantly lower than that in the control group. Compared with the control group, Tartrate-resistant acid phosphatase staining showed lower osteoclast number in Mg, VC, and Mg+VC groups, with the lowest in the Mg+VC group. Osteoblast surface was larger in the Mg and VC groups than in the control group. The bone formation marker PINP level in the VC and Mg+VC groups was significantly higher than that in the control group. The bone resorption marker CTX level was significantly lower the Mg+VC group than in the control group. Immunohistochemistry staining showed lower TNFα level in bone marrow in the Mg, VC, and Mg+VC groups than in the control group. Angiography showed significantly more blood perfusion in the proximal tibia and significantly fewer leakage particles in the distal tibia in the Mg and Mg+VC groups than in the control group at week 2. Micro-CT showed significantly better trabecular architecture in the VC and Mg+VC groups than in the control group at week 6.

**Conclusion:** Oral Mg and VC supplementation alleviated SAON in rats, and the combination of Mg+VC was most effective.

**FP5.7**

**Dexamethasone Exerts Concentration-dependent Effects and Skews Cell Fate during Osteogenic Differentiation of Mesenchymal Stem Cells: A New Finding in the Molecular Pathogenesis of Glucocorticoid-induced Osteoporosis**

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**FP5.8**

**Ginkgo Biloba Ameliorates Postmenopausal Osteoporosis by Rebalancing Bone Metabolism: A Preclinical Study**

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Osteoporosis contributes to the progressive loss of physical autonomy in the postmenopausal population. Bone remodelling includes consecutive bone resorption and bone formation, and such processes are coordinated by bone-resorbing osteoclasts and bone-forming osteoblasts. Tilt of this balance towards bone resorption in postmenopausal women is associated with loss of bone mass and the deterioration of micro-architecture, which collectively result in the compromise of structural strength. Current anti-osteoporotic medications either stimulate bone formation or inhibit bone resorption. Natural herbal extractions are used for treating various degenerative conditions. In particular, ginkgo biloba extract (GBE), a phytochemical product from ginkgo biloba leaf, has demonstrated its therapeutic efficacy on a number of ageing-related diseases. In the present study, we found that oral gavage of GBE (400 mg/kg) in C57BL/6J ovariectomised (OVX) mice improved bone mineral density (1.27 ± 0.01 mg/cm²) compared with the control group (1.23 ± 0.02 mg/cm²). Moreover, we identified that one of the GBE components, GBE-X (12 mg/kg), is sufficient to significantly increase bone mineral density in OVX mice. The GBE-X can also significantly improve the trabecular bone micro-architecture as revealed by increases of BV/TV by 68% and BS/TV by 63%, and a decrease of BS/BV by 33%. The GBE-X promoted bone remodelling by reversing the serum OPG-to-RANKL ratio and prevented OVX-induced osteoporosis by simultaneously promoting osteogenesis and repressing osteoclastogenesis. Together, our results indicate that GBE ameliorates osteoporosis by rebalancing bone metabolism. Further clinical translation is warranted.

**FP5.9**

**Composite Model to Predict Curve Severity of Adolescent Idiopathic Scoliosis: A 6-Year Longitudinal Study beyond Skeletal Maturity**

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**Introduction:** The aetiopathogenesis of adolescent idiopathic scoliosis (AIS) remains unclear. Early risk evaluation of curve severity for timely intervention is important. We previously reported miR-145-5p in AIS osteoblasts impairing osteocytogenic differentiation and we have discussed the clinical implication of serological markers in a small sample size cohort. In the present study, we aimed to evaluate the prognostic value of a composite model in predicting curve severity.

**Methods:** This was a 6-year longitudinal cohort study. Girls with AIS (n=100) were recruited at their first visit to our clinic. We took blood samples and measured anthropometry and curve severity. The patients with AIS were followed up clinically every 6 months for 6 years. Severe AIS was defined as Cobb’s angle >40°. Serum CTX/P1NP and plasma miRNA level were determined.

**Results:** Thirty patients had severe AIS at the end of the 6-year study. Patients with severe AIS had significantly higher serum levels of P1NP and significantly lower plasma levels of miR-145 compared with patients with AIS with Cobb’s angle <40°. Plasma miR-145 levels showed negative correlation with Cobb’s angle (p=0.002). Using clinical anthropometric parameters, serum P1NP, and plasma miR-145, our composite model showed outstanding power to predict curve severity with $R^2$ 0.575 and hazard ratio of 14.506.

**Conclusion:** The composite model constructed in this study can guide decision making and disease management at an early stage of AIS to help prevent progression of spinal deformity.
FP5.10

Three-dimensional Acetabular Morphology of Chinese Population: A Computed Tomography-based Study

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Introduction: Acetabular morphology plays an important role in the development of various hip pathologies and the design for total hip arthroplasty prostheses. We studied the three-dimensional acetabular morphology in a Chinese population and compared our results with existing data from Caucasian populations.

Methods: Volumetric computed tomography pelvis data of 57 individuals (24 male, 33 female) without hip symptoms were utilised for three-dimensional construction. Acetabular sphere was established. Intersection arcs of 35 planes and acetabular sphere were defined. Angles of the arcs uncovered by the acetabulum were measured. Such angles reflect the morphological features of acetabular rim. The most prominent and depressive part of acetabular rim can be represented as the smallest and largest angle.

Results: All angles of the arc uncovered by the acetabulum were >90°, meaning the Chinese acetabulum is a sub-hemispherical structure. The acetabular rim has three prominences and two depressions. The peak of the anterosuperior prominence is located at 58.3°, the peak of the anteroinferior prominence is located at 139°, the peak of the posteroinferior prominences is located at 234.3°, the peak of the anterior depression is located at 102.3°, and the peak of the posterior depression is located at 323.1°. There were no significant difference between male and female acetabula (p>0.05). When compared with the Caucasian data, Chinese acetabula are smaller and shallower.

Conclusion: The complex geometry of the concave acetabulum benefits from three-dimensional visualisation and quantification. A shallow acetabulum may be the cause of low incidence of pincer-type femoroacetabular impingement and primary osteoarthritis. It may also call for the need of special Asian prostheses in total hip arthroplasty.

FP5.11

Histological and Biomechanical Study of All-suture Anchor versus Traditional Transosseous Repair on a Rat Rotator Cuff Tear Model

Y Liu, HT Leong, SC Fu, PSH Yung

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FP5.12

Effect of Differentially Expressed LBX1 in Adolescent Idiopathic Scoliosis Paraspinal Muscle Phenotypes via Modulating Myoblasts

YJ Wang, KL Cheng, TP Lam, ALH Hung, JCY Cheng, WYW Lee

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