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Abstracts of Oral Contributions

OCO1

Objectively recorded minutes of moderate and vigorous physical activity and impact counts of peak accelerations predict variance in bone strength at the weight-bearing tibia but not the non-weight-bearing radius in children

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The Canadian 24-Hour Movement Guidelines for Children and Youth recommend at least 60 min/day of moderate to vigorous physical activity (MVPA), as well as vigorous activity (VPA) and muscle and bone strengthening activities at least 3 times/week. However, the type, intensity and duration of activities that strengthen bone remain unclear. Our objective was to assess if minutes of MVPA, VPA and estimated bone impacts would independently predict variance in radius and tibia bone strength in children. We recorded 7-day physical activity of 49 children, mean age=11 (SD 1.7y), using accelerometers and estimated bone strength at the radius and tibia using peripheral quantitative computed tomography. We assessed if daily average MVPA, VPA, and peak acceleration impact counts (≥3.9 g resultant magnitude) would predict variance in bone strength after adjusting for sex, body mass and site-specific muscle area. We report significant (P<0.05) standardized β-coefficients and R2 of the adjusted linear regression models. MVPA mean 50.2 (SD 22.6) min/day, VPA 18.6 (10.6) min/day, and impacts 70.9 (59.4) counts/day did not predict variance in bone strength at the radius. VPA (β=0.24, R2=0.55) predicted variance in distal tibia strength. MVPA (β=0.20, R2=0.71), VPA (β=0.24, R2=0.72), and impacts (β=0.21, R2=0.71) predicted variance in tibia shaft strength. Based on our models, a 10-minute increase in MVPA or VPA could increase tibia bone strength by 3-7%. An increase of 63 impact counts/day could similarly increase tibia bone strength by 7%. Our predictions and objectively quantified activities associated with forearm strength need to be assessed in future intervention studies.

OCO2

Bone and muscle strength, physical activity and animal-assisted-intervention to increase physical activity in children with autistic spectrum disorder

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Introduction: Increased fracture incidence in individuals with Autistic Spectrum Disorder (ASD) has been associated with poor bone strength and low physical activity. Our objectives were to: 1) compare bone and muscle strength and physical activity between children with and without ASD; and 2) assess the effect of therapy-dog intervention to increase physical activity in children with ASD.

Methods: We recruited 21 children (17 boys) mean age 10.3, SD 2.6 yrs with ASD and 167 typically-developing children (87 boys), mean age 10.7, 1.9 yrs. We scanned the radius and tibia using pQCT to estimate bone strength and muscle areas. We recorded grip force and long-jump length and measured daily minutes-of-moderate-to-vigorous-physical-activity (MVPA) from 7-day accelerometer wear. We compared adjusted (sex, age, height and weight) bone and muscle outcomes and mean MVPA between ASD cases and controls. Children with ASD participated (1 session/week) in physical activity intervention for 8 weeks and we used cross-over design to test if MVPA differed between randomized sessions with or without therapy-dog.

Results: Children with ASD had 24% and 17% lower adjusted bone strength in the radius and tibia shafts (p<0.001). Leg muscle area was 20%, grip force 17% and long jump length 32% lower in children with ASD (p<0.001). Minutes of daily MVPA did not differ between the groups. Therapy-dog intervention did not increase minutes of MVPA in children with ASD.

Conclusions: Findings suggest that physical activity interventions focused on muscle strengthening rather than MVPA may benefit bone strength development and fracture prevention in children with ASD.

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OC03

Sclerostin-neutralizing antibody treatment increases *Serpinf1 (PEDF)* expression in mice tibiae independent of mechanical loading

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Bone adapts to mechanical stimuli to maintain an optimized structure. Mechanical loading causes decreased expression of sclerostin, an osteocyte-specific secreted glycoprotein encoded by the Sost gene, known to inhibit Wnt-ß-catenin signaling. Previous microCT analyses (1) of 78 wk old mice treated with sclerostin neutralizing antibody (Scl-Ab) and loaded for 2 wks showed a bone formation response. Recent analyses of these data using time-lapse microCT registration (2) showed an additive formation response to loading and Scl-Ab. We aimed to examine the effect of load on gene expression after acute sclerostin inhibition. We performed a single bout of *in vivo* cyclic compressive loading to the left tibia of 78 wk old female C57Bl/6J mice, half of which treated with Scl-Ab. Mice were dissected at various times after loading. RNA analyzed by microarray/qPCR. *Serpinf1* was found to be in the top ten regulated genes following Scl-Ab treatment (Fig. 1A). Serpinf1 expression levels were highly upregulated independent of loading. *Sost, Dkk1, Wif1* (Fig. 1B) were also highly upregulated in response to Scl-Ab. Results were validated by IHC. Other recent studies have shown Scl-Ab treatment causes activation of Wnt target genes (3,4). *Serpinf1* and/or other Wnt pathway inhibitors might be responding to decreased tissue level strains due to increased bone mass. *Serpinf1* may also be upregulated due to increased mineralization (5), although we saw unchanged serpinf1 expression after 2wks of load-induced bone formation in vehicle treated group. These data provide a possible explanation for the diminished bone formation response to Scl-Ab after longer-term treatment (6).

OC04

Mutations in fibronectin cause a subtype of spondylometaphyseal dysplasia with "corner fractures"

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Fibronectin is a master organizer of extracellular matrices (ECM), promoting assembly of collagens, fibrillin-1, and other proteins. It is also known to play roles in skeletal tissues through its expression in osteoblasts, chondrocytes and mesenchymal cells. Spondylometaphyseal dysplasias (SMD) comprise a diverse group of skeletal dysplasias often presenting with short stature, growth plate irregularities, and vertebral anomalies, such as scoliosis. By comparing the exomes of individuals with SMD with the radiographic appearance of "corner fractures" at metaphyses, we identified three individuals with novel variants in the fibronectin gene (FN1) affecting highly conserved residues. Furthermore, using matching tools and the SkelDys emailing list, we identified other individuals with de novo FN1 variants and a similar phenotye. The severe scoliosis in most individuals and rare developmental coxa vara distinguishes individuals with FN1 mutations from those with classical Sutcliffe type SMD. To study functional consequences of these FN1 mutations, we introduced three disease-associated missense mutations (p.Cys87Phe, p.Tyr240Asp, p.Cys260Gly) in a recombinant secreted N-terminal 70 kDa fragment (rF70K) as well as in the full length fibronectin (rFN). The wild-type rF70K fragment and rFN were secreted into the culture medium, whereas all mutant proteins were either not secreted or secreted at significantly lower amounts. Immunofluorescence analysis demonstrated increased intracellular retention of the mutant proteins. In summary, mutations in FN1 that cause defective fibronectin secretion are found in SMD, and we thus provide additional evidence for a critical function of fibronectin in cartilage and bone.

OC05

Neuronal loss of the osteopetrotic *Ostm1* gene leads to neurodegeneration and motor disease

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Mutations in the human and mouse *OSTM1/Ostm1* gene result in the most severe form of autosomal recessive osteopetrosis, an inherited bone disorder resulting from defective hematopoietic osteoclasts. We isolated and characterized the *Ostm1* gene responsible for the spontaneous murine osteopetrotic *gl* mutation. Functional rescue of hematopoietic defects was obtained in *gl/gl*-PU.1-*Ostm1* BAC transgenic mice. These *gl/gl* transgenic mice demonstrated however severe gliosis, neurodegeneration with impaired autophagy and became overtly ill and died prematurely around 6-7 weeks. To investigate whether Ostm1 has a direct role in neuronal cells, two complementary approaches were undertaken. Transgenic mice expressing *Ostm1* under the control of the Synapsin-1 promoter were first generated and the bigenic Synapsin1-*Ostm1*/PU.1-*Ostm1* mice on a gl/gl background displayed full phenotypic rescue with normal life span, suggesting a major role of Ostm1 in neuronal physiology. Secondly, we generated a conditional *Ostm1*$^{lox/lox}$ allele to address the *Ostm1* neuronal specificity with Synapsin-Cre ablation. These neuronally ablated Synapsin1-Cre-*Ostm1*$^{lox/lox}$ mice develop a rapid and progressive deficits starting around ~9-10 weeks of age. Behavioural study of these mice show abnormal limb-clasping reflexes, tremor and gait defect. Consistently, patho-histology revealed loss of neuronal cells in CA3 and dentate gyrus regions of the hippocampus, thinning of the cortex and severe gliosis. End-stage disease is characterized by hind limbs paralysis associated with muscle fiber atrophy and denervation of neuromuscular junctions. Together, our data provide evidence that the Ostm1 gene plays a crucial role in neuronal homeostasis independently of the hematopoietic lineage and further establish a key Ostm1 bone-brain-muscle interaction network.

OC06

The effects of glucocorticoids and vitamin D deficient diet on musculoskeletal health in growing Mdx mice

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Duchenne Muscular Dystrophy (DMD) patients suffer both muscle degeneration and inflammation, as well as bone fragility. As insufficient levels of vitamin D (VD) are consistently found in DMD boys, this study examined the effects of VD deficiency and glucocorticoids, the standard therapy for DMD, on musculoskeletal system using the Mdx mouse model of DMD during rapid growth. Four-week old Mdx mice were fed either control (1.0 IU/g) or vitamin D deficient (0.1 IU/g) diet. At 5 week-old, some mice received a slow-release pellet of prednisone (5 mg for 60 days). To examine the intrinsic effects of dystrophic muscle on bone, Mdx mice were compared to wild-type mice. Mice were sacrificed at ten week-old when strain and treatment effects were assessed by one-way ANOVA. The presence of dystrophic muscle reduced cortical bone stiffness and also resulted in osteopenic trabecular bone in femurs (32% vBMD decrease and 67% reduction in bone volume) with increased osteoclasts in growing Mdx mice. Glucocorticoids retarded body and bone growth and induced cortical bone loss by suppressing osteoblasts and bone formation rate, but improved muscle function. VD deficiency alone did not have a significant effect on growing bone and muscle in Mdx mice. The combination of glucocorticoid and low VD increased cortical bone elasticity, suggesting an effect on bone collagen. This study highlighted the detrimental effects of dystrophic muscle on growing bone, which is further exacerbated by glucocorticoid treatment. VD deficiency with or without glucocorticoids did not exert additional detrimental effects on growing bone and dystrophic muscle in Mdx mice.

OC07

Prophylactic bisphosphonate therapy to reduce glucocorticoid-induced osteoporosis in a mouse model of Duchenne Muscular Dystrophy

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Patients with Duchenne Muscular Dystrophy (DMD) have poor bone health partly due to the underlying disease and partly due to treatment with glucocorticoids (GCs). Bisphosphonates such as pamidronate (PM) and zoledronate (ZA), are used to treat children at risk for bone fractures. **Objective:** To explore if prophylactic treatment with different types of BP ameliorate GC-induced bone loss in a murine model of DMD. **Methods:** 4 week-old *mdx* mice, a commonly used DMD animal model, were injected subcutaneously with vehicle (saline) or 8 mg/kg PM or 0.08 mg/kg ZA over 2 weeks or 8 mg/kg PM over 6 weeks. At 5 weeks-old, animals received either placebo or 5 mg prednisone slow-release pellets. WT (C57BL/10ScSn) mice were implanted with placebo pellets and injected with saline. Weight and forelimb grip strength was assessed weekly. Mice were sacrificed at 10 weeks-old and tissue samples taken to assess bone loss and muscle degeneration. **Results:** GC increased muscle strength in *mdx* mice and this was not affected by PM or ZA treatments. Both PM and ZA treatments significantly increased bone mineral density (BMD) and cortical bone area compared to GC saline-treated *mdx* mice. PM and ZA treatments significantly increased femur trabecular vBMD and trabecular bone volume to levels greater than mdx or WT controls. Preliminary data suggest that at the doses given, PM treatments have greater effect on trabecular bone compared to the ZA treatment. Samples are currently being assessed for histomorphometric changes that can account for changes in bone microarchitecture and bone biomechanical properties.

OC08

Mechanical loading maintains bone mass *in vivo* in osteolytic multiple myeloma

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Multiple myeloma (MM) is an incurable plasma cell derived neoplasia, leading to pathological osteoclast activity resulting in increased bone resorption. Treating MM bone disease is a major goal in MM therapy. We hypothesized, that loading would maintain bone mass in a mouse model of MM. The left tibiae of 40, 10wk old female BALB/c mice were either a) PBS injected, b) MOPC315.BM cell injected (2), or c) not injected. 14 days after injection, 25 mice (n = 7-10/treatment) underwent 3 wks of *in vivo* tibial loading (3) (left limb, right limb nonloaded), with the remaining 15 mice (n = 6 PBS, n = 9 tumor) serving as nonloaded controls. *In vivo* microCT was performed at day 0, 5, 10, 15 and 20 of loading, at proximal metaphysis and midshaft. Cortical and trabecular parameters were measured. An ANOVA assessed the effects of treatment, loading, and limb. After 20 days of loading, metaphyseal cortical bone formation (CtTh, CtAr, TAr, CtAr/TtAr, CtvTMD) was significantly greater and cortical porosity (CtPo) was lower compared to nonloaded right limbs and nonloaded groups. All right limbs had similar increases in cortical parameters over 20 days, indicating no systemic effects present. Left limbs of tumor-injected loaded group had + 41% cortical thickness, while left limbs of tumor-injected nonloaded group increased by 1%. In conclusion, these data indicate mechanical loading can maintain bone mass in this model of MM.

1) Lynch et al. JBMR 2013. 2) Hofgaard et al. PLoS One 2012. 3) Willie et al. Bone 2013.

OC09

Osteolysis, pain and sensory neuroplasticity due to experimental breast cancer bone metastases

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Skeletal complications occur in 70% of patients with advanced breast cancer and include fractures, hypercalcemia and severe bone pain. Increased understanding of bone and sensory alterations is required to improve disease pain management. The

goal of our study was to characterize behavioral pain phenotype in experimental breast cancer bone metastasis model and to explore the pain-relieving effects of microenvironment-targeting adjuvant therapies. Immunocompetent BALB/c mice were injected intra-tibially with murine mammary carcinoma cells (4T1) or saline (sham). Gradual development of osteolysis was observed in the cancer-injected limbs starting at 2-3 weeks post-injection. Sensitivity to mechanical, heat and cold stimuli in cancer-bearing and contralateral limbs, as well as spontaneous limping and guarding significantly increased in cancer-bearing animals compared to sham 3 weeks following inoculation. Histomorphometry demonstrated systemic osteoclast activation and bone loss in cancer-bearing animals. The expression of calcitonin gene related peptide (CGRP) in sensory neurons and glial fibrillary acidic protein (GFAP) in spinal cords significantly increased in tumor-bearing animals both ipsilateral and contralateral to tumor inoculation. The anti-inflammatory and osteolysis-targeting drug rapamycin reduced hypersensitivity to mechanical and thermal stimuli in the cancer-bearing and contralateral limbs, attenuated GFAP over-expression and lowered osteoclast number. The osteoclast-targeting drug pamidronate reduced thermal sensitivity at the cancer-bearing and contralateral limbs and protected against bone loss. Thus, localized bone cancer drives osteolysis, sensory hypersensitivity, and neuroplasticity both locally and distantly from the primary lesion. Drugs targeting these mechanisms may be useful in the treatment of pain associated with breast cancer bone metastasis.

OC10

Identification of genetic markers for early detection of skeletal muscle function deficits in childhood acute lymphoblastic leukemia survivors

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Although 80% of childhood acute leukemia (ALL) cases are cured with current treatment protocols, exposure to chemotherapeutics or radiation therapy during a vulnerable period of child development has been associated with high frequency of late adverse effects (LAE). Previous observations suggest important skeletal muscle function, size and density deficits in ALL survivors. Given that only a fraction of all patients will suffer from these treatment-related complications, we interrogated whether they could be predicted by a cluster of genetic markers. To this end, we analyzed in 244 childhood ALL survivors an association between skeletal muscle force (Fmax) and power (Pmax) and genetic variants from 1039 genes derived through whole-exome sequencing (WES). Top-ranking association signals retained after correction for multiple testing were confirmed through genotyping, and further analyzed through stratified analyses and multivariable models. Our results show that muscle function deficit is associated with two common SNPs (rs2001616DUOX2, p=9x10-5 and rs41270041ADAMTS4, p=0.04) and two rare ones located in the ALOX15 gene (p=10-4). These associations were further modulated by patients' demographics and treatment features. Occurrence of muscle function deficits in childhood ALL is strongly modulated by variations in the DUOX2, ADAMTS4 and ALOX15 genes, which might be applicable for personalized prevention strategies in childhood ALL survivors.

OC11

Targeting the muscle-bone unit in a model of severe osteogenesis imperfecta: effect of activin receptor 2b inhibition

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Objective: OI is mainly characterized by bone fragility but also by reduced muscle mass and function. Muscle mass and bone mass are closely linked, wherefore an intervention that increases muscle mass should also increase bone mass. Here we investigated the effect of a novel GDF ligand trap (Acceleron Pharma) on skeletal muscle mass and bone properties in a mouse model of severe dominant OI, the Col1a1Jrt/+ mouse.

Methods: Starting at an age of 8 weeks, GDF ligand trap (3 mg or 10 mg per kg body mass) or vehicle was injected subcutaneously twice per week for 4 weeks into male OI and wild-type (WT) mice.

Results: At baseline, OI mice had 20% lower body mass than control littermates. This difference persisted as OI and WT cohorts exhibited a similar dose-dependent increase in body mass during treatment. In WT and OI, intervention led to a dose-dependent increase in muscle mass of quadriceps and gastrocnemius by about 36% and 50%, respectively. In WT, soleus and EDL weights were also increased in a dose-dependent manner, whereas in OI weights of soleus and EDL were increased as well but without dose-effect. Concerning bone unit, intervention significantly improved femoral trabecular bone volume in WT mice only. However, in OI mice intervention resulted in significantly improved femoral length and significantly increased mid-diaphyseal periosteal diameter.

Conclusion: GDF ligand trap increases muscle mass and improves diaphyseal bone geometry in a model of severe OI, representing a new therapy option for severe OI.

OC12

Metabolic phenotype in the mouse model of osteogenesis imperfecta

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Osteogenesis Imperfecta (OI) is the most common heritable bone fragility disorder, usually caused by dominant mutations in genes coding for collagen type I alpha chains, COL1A1 or COL1A2. Osteocalcin is now recognized as a bone-derived regulator of insulin secretion and sensitivity and glucose homeostasis. Since OI is associated with increased rates of bone formation and resorption, we hypothesized that the levels of undercarboxylated osteocalcin are increased in OI. The objective of this study was to determine changes in osteocalcin and to elucidate the metabolic phenotype in the Col1a1Jrt/+ mouse, a model of dominant OI caused by a Col1a1 mutation. Circulating levels of undercarboxylated osteocalcin were higher in 4-week old OI mice and normal by 8 weeks of age. Young OI animals exhibited a sex-dependent metabolic phenotype, including increased insulin levels in males, improved glucose tolerance in females, lower levels of non-fasted glucose and low adiposity in both sexes. The rates of O2 consumption and CO2 production, as

well as energy expenditure assessed using indirect calorimetry were significantly increased in OI animals of both sexes, while respiratory exchange ratio was significantly higher in OI males only. While OI mice have significant physical impairment that may contribute to metabolic differences, we specifically accounted for movement and compared OI and WT animals during the periods of similar activity levels. Taken together, our data strongly suggest that OI animals have alterations in whole body energy metabolism that are consistent with the action of undercarboxylated osteocalcin.

OC13

Hematopoietic cell transplantation and enzyme replacement therapy rescue muscle and bone weakness in Hurler mice

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Mucopolysaccharidosis type I-Hurler is caused by a deficiency of a-L-iduronidase, leading to a progressive disorder with multiple tissue involvement including musculoskeletal deformities. Currently there is no cure. To determine efficacy of hematopoietic cell transplantation (HCT) and enzyme replacement therapy (ERT) on musculoskeletal function, 52 IDUA knock-out mice were randomly assigned: PBS only (PBS), HCT plus PBS (HCT), HCT plus low (LOW) or high dose enzyme (HIGH). Wild-type C57BL/6 mice (WT) served as controls. HCT was performed at 4-5 wks after birth and ERT was delivered i.v. weekly for 11 mo. *In vivo* hindlimb muscle contractility measured at 9-10 mo of age showed that peak isometric torque was different among groups (p=0.002) with WT, HCT and HIGH mice generating more torque than PBS mice. Passive torque required to dorsiflex the ankle of LOW mice was also lower compared to PBS mice (p=0.034). Mechanical testing of tibia showed that PBS mice had lower ultimate stress and normalized modulus of elasticity compared to WT and that HCT and ERT improved these mechanics in PBS mice ($p \leq 0.005$). The functional improvements appear to be driven by changes in cortical cross-sectional moment of inertia as measured by uCT. However, there were no treatment effects on ultimate load or stiffness ($p \geq 0.055$). In conclusion, HCT plus ERT improved the muscle and bone weakness of Hurler mice and guide us toward therapeutic strategies. Supported by NIH T32-AR050938, Shriners Children's Hospital and Genzyme, Inc.

OC14

Milk and alternatives intervention and bone and muscle mass accretion in 14 to 18 y postmenarcheal girls: Preliminary results at 12 months from a 2-year randomized controlled trial

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Canadian adolescents do not meet Canada's Food Guide recommendations for Milk and alternatives (MAlt) despite its important role in optimizing bone and muscle health. We examined whether increased MAlt intake over 12-mo altered bone and muscle accretion in girls with usual intake of <2 servings of MAlt/d. Adolescents (14 to 18 y) from Greater Montreal participating in a trial (NCT02236871) of control [no intervention], improved (IInt) [2 to 3 MAlt servings/d] or recommended (RInt) [3 or more MAlt/d) intervention groups were studied. Trabecular and cortical volumetric bone mineral density, cortical thickness and bone area of the radius (4% and 66% sites), the tibia (4%, 38% and 66% sites) and muscle cross-sectional area (mCSA) and density (66% sites) were assessed at baseline (BL) and 12 mo using peripheral quantitative computed tomography (XCT-2000; Stratec) along with anthropometry and 24-hour recalls. Differences among groups were tested using mixed model ANOVA with post-hoc Bonferroni adjustment. Data are mean±SD unless otherwise stated. At BL, groups ((mean age 16.2±1.5, n=27) were not different in any of the measurements. At 12 months, bone parameters did not differ among groups, whereas the radial mCSA was significantly higher in the RInt group compared with the control (p=0.0008) and the IInt (p=0.005) groups. The RInt groups had greater increases in radial mCSA compared with the control and the IInt groups (+43.8% vs +2.1% vs +2.5% respectively, p<0.02). Increasing MAlt intake to meet recommendations appears to favor radial muscle accretion.

OC15

Muscle Function Tests as Field Measures of Tibial Bone Strength

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Optimizing bone strength during adolescence may reduce stress fracture in the short term and fracture later in life. However, the complexity of measuring bone strength creates a practical problem for health professionals. The muscle-bone relationship consistently reflects the association between muscle strength changes and bone strength changes in response to exercise at all ages Therefore, the purpose of our study was to identify the muscular fitness measure (relative grip strength, 1 RM leg extension or peak power calculated from a vertical jump) that was most predictive of bone strength in a healthy population. **Methods:** 55 participants, 28 females and 27 males (age (yrs) 28.5 +/- 10.0, height (m) 1.7 +/- 0.8, body fat % 25.8 +/- 10.1) performed a relative grip strength test using a hand dynamometer, a one repetition maximum (1 RM) on a leg press machine and a vertical jump test using the Vertec. Peak power was then calculated from vertical jump height. Moment of inertia (J), cortical area (Ct.Ar.), cortical bone mineral density (cBMD), and strength-strain index (SSI) were measured using peripheral Quantitative Computed Tomography (pQCT) to determine bone strength at the 50% tibia site. **Results:** 1 RM/BW was not correlated and relative grip strength was weakly correlated with bone strength parameters. Peak power resulted in significant, positive correlations with J (r=0.6902, p<0.0001), Ct.Ar. (r=0.7454, p<0.0001), and SSI (r=0.6764, p<0.0001). Cortical BMD was not correlated with peak power. Our findings suggest peak power was a significant surrogate measure of bone strength in a healthy population.

Abstracts of Poster Presentations

POO1

Muscle-bone interactions in Chinese men and women aged 18 to 35 years

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Little is known about muscle-bone relationships in Chinese men and women, and whether these relationships change relative to the

achievement of peak bone mass. This study examined differences in bone density and lower body strength and power in Chinese adults (n=53) residing in the US for <5 yrs. Subjects were grouped by sex (male (n=28) and female (n=25)) and age (bone accruing: 18-25 yrs (n=30) and peak bone mass: 26-35 yrs (n=23)). Questionnaires assessed diet and physical activity. DXA assessed aBMD and body composition and pQCT assessed cortical and trabecular bone of the non-dominant tibia. Vitamin D concentrations were measured by ELISA. Leg muscle power was assessed by a jump mat and leg strength with a two-leg press maximal strength test. As expected, there was a significant (p=0.001) sex main effects for most variables, however, no significant age group effects or sex age group interactions were found. There were no differences between groups for calcium intakes, vitamin D levels, and vBMD at the 38% and 66% tibial sites. Most correlations, regardless of sex or age group, for measures of strength, power, body composition, and bone measures were moderately strong and ranged between r=0.40 to r=0.80. In conclusion, Chinese men had greater measures of strength, power, and indicators of bone health compared to Chinese women, although relationships between variables were similar for both sexes and age groups. There were no differences in our outcome variables between individuals accruing bone (18-25 yrs) and those at peak bone mass (26-35 yrs).

P002

Peripheral quantitative computed tomography imaged measures of muscle area and density at the forearm and lower leg of children are precise with errors ranging between 1-4%

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Introduction: Muscle area and density are commonly measured using peripheral quantitative computed tomography (pQCT) in pediatric musculoskeletal studies. However, the repeatability of reported image analyses is unknown.

Objective: To define and compare pQCT precision errors and least-significant-changes (LSCs) for muscle outcomes at the forearm and lower leg in children using two image analysis thresholds.

Methods: We obtained duplicate pQCT scans (>24 hrs apart) of forearm and lower leg from 35 children (13M:22F; mean age 10.5, SD1.7 yrs). We separated muscle tissue from subcutaneous fat using a threshold of 30 or 40 mg/cm^3, and from bone using a threshold of 280 mg/cm^3. We report root-mean-squared coefficient of variation (CV%$_{RMS}$) and LSCs for muscle area and density at both sites. We compared precision errors obtained with two thresholds using paired t-test.

Results: At the forearm, precision errors were 2.6% (30 mg/cm^3) and 2.8% (40 mg/cm^3) for muscle area, and 1.9% (30 mg/cm^3) and 2.3% (40 mg/cm^3) for density. At the lower leg, precision errors were 3.5% (30 mg/cm^3) and 3.6% (40 mg/cm^3) for area, and 1.1% (40 mg/cm^3) and 1.4% (30 mg/cm^3) for density. There was no significant differences in precision errors between thresholds (p>0.05). LSCs indicated an observed 8-10% change in area and 3-7% change in density needed for reliable detection of muscle changes in both limbs (analyzed with either threshold).

Conclusion: Precision errors for pQCT-measured muscle area and density in the forearm and lower leg of children were low (CV%$_{RMS}$ <4%) and comparable for both thresholds tested. Contrasting observed errors to prospective changes in children's muscle outcomes is warranted.

P003

Normative data for lean mass in healthy term infants from 1 month to 1 year of age

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Dual-energy X-ray absorptiometry (DXA) has made it possible to assess body composition in pediatrics. However, there is a dearth of reference data for lean mass (LM) in infancy despite the growing evidence that a leaner body phenotype in infancy plays an important role in early life prevention of obesity. This prospective study was conducted to assess LM of 71 healthy, term and breastfed infants followed from ≤1 month until 12 months of age. Infants (36 boys; 35 girls) with 25-hydroxyvitamin D [25(OH)D] values of ≥50 nmol/L from Montreal, Quebec Canada, were recruited between March 2007 and August 2010. LM (g) was measured using DXA (APEX version 13.2, Hologic 4500A) in infant whole-body mode. Plasma 25(OH)D concentration was measured by liquid chromatography tandem mass spectrometry. Infants' growth was healthy according to the World Health Organization standards and doubled between baseline and 12 months follow-up (4638.7±729.1 vs 9711.0±1200.9 g). There was steady LM accretion (mean±SD): ≤1 mo: 3778.8±473.2; 3 mo: 4488.8±639.4; 6 mo: 5404.9±831.4; 9 mo: 6408.4±940.9; 12 mo: 7346.9±1169.1 representing a 94% increase in LM. Boys had more LM compared to girls over the study (5555.57±1572.31 vs 4966.85±1367.78 g). Percent LM decreased between ≤1 and 6 mo (77.3±6.3 vs 68.3±9.2%) and increased between 6 and 12 mo (68.3±9.2 vs 74.7±7.8%). This data, which is based on a healthy sample of infants, characterises LM accretion during the first year of life and will aid in the interpretation of DXA scans for body composition by health professionals and researchers.

P004

Can we reliably monitor muscle force development in children?

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Introduction: Muscle forces provide the largest voluntary loading on bone. Reliable monitoring of muscle force development is therefore fundamental in pediatric bone studies. We aimed to assess reliability of muscle force measurements in children by: 1) defining precision errors and 2) contrasting these errors to annual muscle force development in children.

Methods: To define short-term precision errors, we measured maximal pushup, grip force, countermovement and long jump peak forces on two different occasions (approximately 1 month apart) from 33 children (18F) with mean age of 10.5, SD 1.8 yrs. To define annual change, we obtained the same measures approximately one year after the first visit from 33 (19F) participants. We report root-mean-squared coefficient of variation (CV%RMS) to define precision errors, and %-changes to define annual change (significance tested using paired t-test, p<0.05) in muscle force measures.

Results: For the pushup force, precision error was 9% and annual

increase 33% (p<0.001). For the grip force, precision error was 14% and annual increase 43% (p<0.001). For the countermovement jump force, precision error was 11% and annual increase 19% (p<0.001). For the long jump, vertical and horizontal force precision errors were 8 and 9% while annual increases were 19 and 24%, respectively (both p<0.001).

Conclusion: Increases in muscle forces over one-year follow-up exceeded related short-term precision errors. These findings indicate that annual muscle force development can be reliably monitored in the upper extremity with push-ups and grip force measurements and in the lower extremity with countermovement and long jumps measurements in children.

P005

Sex- and ethnic-specific reference curves for bone microarchitecture, density and strength using high-resolution peripheral quantitative computed tomography in 9 to 20 year olds

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Normative high-resolution peripheral quantitative computed tomography (HR-pQCT) data do not currently exist for children and adolescents. Thus, we aimed to develop site-, sex-, ethnic-, and age-specific reference curves for HR-pQCT in 9-20 year olds. We acquired annual HR-pQCT scans (XtremeCT, Scanco Medical) at the distal radius (7% site) and tibia (8% site) in a convenience sample of 354, 9-21-year-old (189 girls, 51% Asian; 165 boys; 50% Asian) participants in the longitudinal University of British Columbia Healthy Bones III Study. We acquired a maximum of four annual measurements (n=1093 observations). We report standard morphological measures: total BMD (Tt.BMD, mg/cm^3), trabecular number (Tb.N, 1/mm), trabecular thickness (Tb.Th, mm), and bone volume ratio (BV/TV). We used an automated segmentation algorithm to separate trabecular and cortical bone to determine: total bone cross-sectional area (Tt.Ar, mm^2), cortical BMD (Ct.BMD, mg/cm^3), cortical porosity (Ct.Po, %), and cortical thickness (Ct.Th, mm). Finally, we applied finite element (FE) analysis to HR-pQCT images to estimate failure load (F.Load, N). We used the lamda, mu, sigma (LMS) method using LMS ChartMaker Light (Version 2.5, The Institute of Child Health, London, UK) to construct LMS tables and reference centile plots. We report sex- and age-specific centiles (3rd, 10th, 25th, 50th, 75th and 97th) for whites and Asians for HR-pQCT bone parameters at the distal radius and tibia. These reference curves can be used by clinicians and researchers to assess bone health and change in bone parameters over time in healthy or clinical cohorts of children and adolescents.

P006

Can we reliably measure growth and development in cortical bone micro-architecture of children using high resolution peripheral quantitative computed tomography?

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Cortical bone properties (e.g., thickness, porosity) at long bone-ends contribute to bone strength and fracture risk in children and are important targets for monitoring. It is unknown if growth and development of these micro-architectural bone properties in children can be reliably measured using high resolution peripheral quantitative computed tomography (HR-pQCT). To determine if real biological change has occurred, observed change can be compared to measurement precision error at the group-level and to least significant change (LSC) at the individual-level. Our objectives were to: 1) characterize annual changes in cortical bone properties in children; 2) contrast changes to precision errors; and 3) assess the proportion of children with changes exceeding LSC. We scanned the distal radius and tibia of 31 healthy children (18 girls) mean age 10.8(SD1.7) at baseline and after 1.2(0.3) years. We looked at cortical thickness (apparent and fine-structured), density, and porosity. We identified mean changes using paired t-tests (P<0.05). We contrasted mean changes to the published pediatric precision errors and identified the proportion of children with observed change exceeding LSC (Kawalilak et al. 2017). Over 1 year, apparent cortical thickness increased (radius:11.0%; tibia:8.5%), fine-structured thickness increased (12.7%;5.3%), density increased (7.6%;4.4%), and porosity decreased (-24.5%;-13.6%). All changes exceeded corresponding precision error (1.5-11%;0.5-6%). The proportion of children with cortical bone change beyond the LSC were: apparent thickness (radius:33%; tibia:65%), fine-structured thickness (60%;42%), density (67%;77%), and porosity (60%;68%). Results indicate that typical growth and development of clinically-important cortical bone properties in children's distal radius and tibia can be reliably measured using HR-pQCT.

P007

Can cortical geometry, longitudinal bone curvature and bone morphology control the *in vivo* tibial stiffness in growing rats?

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Bone morphology, composition, strength and tissue material properties modulate stresses and strains, which will develop under applied loads and which can influence skeletal physiology and remodeling. Understanding the relationship between applied loads and resulting stresses/strains is crucial for implementing biophysical strategies ensuring healthy bone growth and preventing bone loss. The goal of this study was to relate the *in vivo* tibial stiffness of the growing tibia to the bone geometry and strength, using a rat model. *In vivo* strain response has been measured for three age groups (4 wks, 8 wks, 12 wks old) (n=18) of Sprague Dawley male rats (0.5 to 3.25 mm haversine cyclic deformation at 2 Hz). Bone strength (Young's modulus and ultimate stress) has been evaluated from three point bending tests. Tibial stiffness was related to bone strength, cortical geometry, longitudinal bone curvature and bone mineral density using micro-CT. Statistical analyses were performed using ANOVA tests (p<0.05). Tibial stiffness was maintained from 4 to 12 wks of age, ranging from 11.48 to 11.70. Bone ultimate stress and Young's modulus increased significantly, while bone mineral density, which also increased, showed no significant difference among the age groups. Cortical area and longitudinal bone curvature increased significantly with aging. Tibial stiffness was maintained during growth, since growth-related increases in bone geometrical and mechanical properties produced counteracting effects on the induced bone stresses. Thus, whole bone morphology, including longitudinal curvature and cortical geometry, contributes to maintaining bone stiffness during growth

and should be considered when evaluating and designing *in vivo* loading studies and biophysical skeletal therapies.

P008

Vitamin D status is positively associated with trabecular volumetric bone mineral density of the distal tibia in 14 to 18 y female adolescents with usual intake of <2 daily servings of milk and alternatives

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Associations among vitamin D status, bone geometry and muscle structure in adolescents is under investigated. We explored relationships among serum 25-hydroxyvitamin D (25OHD) and bone and muscle parameters in girls with usual intake of <2 servings of milk and alternatives (MAlt)/d. Adolescents (14 to 18 y) from Greater Montreal participating in a trial (NCT02236871) were divided into three groups according to baseline serum 25OHD concentrations (<50, n=15; 50-74.9, n= 25; ≥75 nmol/L, n=10). Trabecular and cortical volumetric bone mineral density (vBMD), cortical thickness and bone area of the radius (4% and 66% sites), tibia (4%, 38% and 66% sites) and muscle cross-sectional area and density (66% sites) were obtained using peripheral quantitative computed tomography (XCT-2000; Stratec). Other measured were: fasting serum 25OHD (Liaison, Diasorin), anthropometry and 24-hour dietary recall. Data are mean±SD. At baseline, adolescents (mean age 16.4±1.5 y, n=50) were only different for 4% tibial trabecular vBMD where the group with >75 nmol/L was, on average, 37.8% greater than the group with <50 nmol/L. After adjusting for age, height, fat and lean mass, ethnicity, and vitamin D intake, the association between 25OHD and bone parameters remained significant for trabecular vBMD of the 4% tibia (R2=0.2, β=1.6, p=0.04). Findings of this study support a beneficial role of vitamin D status in skeletal health of female youth.

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P009

Non-invasive estimates of bone mechanical properties in children are repeatable: Precision errors range between 3-5%

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Introduction: High-resolution peripheral quantitative computed tomography (HR-pQCT) based finite element (FE) modeling can be used to non-invasively monitor estimates of bone mechanical properties (i.e., bone strength) in children (Gabel et al. JBMR 2017). Information of repeatability (i.e., precision errors) is important when interpreting observed changes and designing pediatric bone studies. Repeatability of bone mechanical properties in children has not yet reported. Our objective was to define precision errors for HR-pQCT FE-derived mechanical properties at the distal radius and tibia in children.

Methods: We scanned distal radius (at 7% site) and tibia (8%) of 32 children (mean age 11.3, SD 1.6 years) twice, 1-week apart, using HR-pQCT. We created linear FE-models by assigning a single elastic modulus (E=6.829 GPa) to bone voxels and applied axial compression. Bone was assumed to be failed when 2% of bone tissue strained beyond 7000 μstrains. We report root-mean-squared coefficients-of-variation (CV%RMS) to define precision errors for the following FE estimates of bone mechanical properties: bone failure load, stiffness, and apparent modulus.

Results: At the distal radius, CV%RMS was 2.8%, 3.3%, and 4.8% for failure load, stiffness, and apparent modulus, respectively. At the distal tibia, CV%RMS was 2.7%, 3.4%, and 4.1% for failure load, stiffness, and apparent modulus, respectively.

Conclusions: Precision errors for the FE-derived bone mechanical properties ranged between 3-5%. Monitoring failure load is recommended with precision errors <3% and reported annual gains of 17% and 12% at the distal radius and tibia, respectively, in children approaching pubertal growth spurt (Gabel et al. JBMR 2017).

P010

Monitoring time interval is less than 1-year for HR-pQCT derived bone mechanical properties in children

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Introduction: High-resolution peripheral quantitative computed tomography (HR-pQCT) based finite element (FE) modeling is a non-invasive method of assessing and monitoring the development of bone mechanical properties. However, annual changes and time required to reliably monitor pediatric bone mechanical properties are unknown. Monitoring time intervals (MTIs) provide the estimated time needed to reliably detect skeletal changes. MTIs guide prospective study design and help avoid unnecessary radiation in pediatric research. Our objectives were to: 1) Assess mean annual changes and 2) define MTIs for pediatric bone mechanical properties.

Methods: We scanned the distal radius (7% site) and tibia (8%) of 39 children (mean age 10.6, SD 1.7 years) twice (1.2, 0.3 years apart) using HR-pQCT. We created FE models using an elastic modulus of 6.829 GPa and applied axial compression. Failure was defined when 2% of bone tissue was strained beyond 7000 mstrain. We report mean annual percent changes assessed using paired t-tests ($p<0.05$) and MTIs for failure load, stiffness, and apparent modulus. We calculated MTIs by dividing least significant changes (based on our lab's pediatric scanning precision errors) by median annual percent changes.

Results: Bone mechanical properties increased 17-18% at the radius and failure load and stiffness increased 12-13% at the tibia ($p<0.05$). MTIs were 0.5 and 0.6 years for failure load, 0.6 and 0.8 years for stiffness, and 0.9 and 1.7 years for apparent modulus, for radius and tibia respectively.

Conclusions: Pediatric bone failure load and stiffness can be reliably monitored every 8 months (radius) and 10 months (tibia).

P011

Changes in bone mass and size in men after completion of growth are characterized by loss of trabecular and cortical bone mineral density but increases in cortical bone size

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Objective: To describe changes in DXA and pQCT-derived bone parameters in a cohort of healthy men over a 12-year follow-up period.

Methods: 428 healthy men aged 25-45 (mean 34.9±5.3) years participated in a longitudinal population-based sibling-pair study; mean follow-up 12.4±0.4 (range 11.2-13.6) years. Areal BMD (aBMD) was measured at the total body, proximal femur and lumbar spine using DXA; trabecular volumetric BMD (vBMD) at the distal radius, cortical vBMD and geometry at the radial and tibial shafts using pQCT.

Results: aBMD decreases were 2.3 ± 3.0% at the total body, 1.7±5.3% at the lumbar spine, 3.1±4.6% at the total hip, 6.0±5.8% at the femoral neck; trabecular vBMD decreased by 1.6±6.5%, cortical vBMD by 0.5±2.7% at the radius and 0.2±1.6% at the tibia. Cortical area, periosteal and endosteal circumference increased by 1.3±6.9%, 5.7±5.9% and 11.9±12.1% at the radius, 1.5±4.2%, 3.3±3.1% and 6.2±7.3% at the tibia; cortical thickness decreased by 5.8±5.6% and 2.5±7.1%. Strength-strain index increases were 3.0±11.4% at the radius and 2.2±5.5% at the tibia. (All p<0.001 except cortical vBMD tibia p=0.047)

Conclusion: In healthy adult men, aBMD as well as trabecular and cortical vBMD start to decrease early after peak bone mass attainment. At cortical bone sites, changes are offset by increases in bone size, leading to an overall increased estimated bone strength.

P012

Relationship between usual milk product intake, whole-body adiposity, bone biomarkers and serum lipids in healthy postmenopausal women

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Emerging evidence suggests that milk product (MP) type may contribute differently to whole-body adiposity, bone health and lipid profile owing to their distinct nutrient matrices. We examined cross-sectional relationships between MP intake (MPi), whole-body adiposity, bone biomarkers and serum lipids in 79 healthy postmenopausal women. We estimated MPi intake (above or below median servings/day) using a 1-month validated food frequency questionnaire. Whole-body adiposity (percent body fat [%BF]) was assessed using bioelectrical impedance analysis, and physical activity (PA) using a validated questionnaire. Fasting serum 25-hydroxyvitamin D (25(OH)D), parathyroid hormone, bone specific alkaline phosphatase, C-terminal telopeptide (CTx), and lipid profile were measured. Differences in participants' characteristics across MPi categories were examined using one-way ANOVA. We estimated associations between MPi and %BF, bone biomarkers, and lipids using multiple linear regression models. There was no difference in participants' characteristics between MPi categories. Median (interquartile range) servings/day of milk, yogurt and cheese were 0.5 (0.1-0.9), 0.4 (0.1.-0.7), and 0.5 (0.3-0.8), respectively. Lower %BF was associated with higher cheese intake (β= -2.75, P=0.05) after adjustment for age, vitamin D intake, and PA. 25(OH)D concentration was positively associated with higher milk intake (β=12.08, P=0.01). Although we found no association between bone biomarkers and MPi, CTx was inversely associated with apolipoprotein B (β=-0.31, P=0.01) in models adjusted for MP type, PA, years since menopause, and %BF. In postmenopausal women, MP types may be differentially linked to whole-body adiposity and bone health. The underlying mechanisms for the inverse association between bone resorption and lipid metabolism require further investigation.

P013

Bone status and circulating micrornas in postmenopausal women

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MicroRNAs (miRNAs) are short, non-coding RNA molecules that fine tune posttranscriptional protein expression. MiRNAs are stable in the blood, therefore, they may be useful as biomarkers of disease. Recently, there is growing interest in the potential clinical utility of circulating miRNAs (c-miRNAs) for osteoporosis patients. This study examined c-miRNAs according to bone status and current hormone replacement therapy use (HRT) in postmenopausal women (n=63), 60 to 85 yrs. Body composition and areal BMD (aBMD) were measured by DXA. C-miRNA expression levels (miR-21, -23a, -24, -100, -125b) were analyzed using real-time PCR, and bone turnover markers were analyzed by ELISA. Sixty-eight% of the women were osteopenic and 13% were osteoporotic, but none of the women had sustained osteoporotic fractures. There was no significant association between bone status and HRT use. There were no significant differences in c-miRNA expression based on HRT use or osteoporosis groups, although miR-21 and miR-23a were differentially expressed (fold change >2) in osteoporotic compared to normal women. MiR-125b was significantly positively correlated with age (r=0.33, p=0.012) and miR-23a was positively correlated with the bone resorption marker (r=0.26, p=0.04). Based on our findings on these 5 selected miRNAs, miR-21 and miR-23a show the most promise as potential biomarkers of osteoporosis status in relatively healthy non-fracture postmenopausal women.

P014

Osteoporosis in postmenopausal women with hypercalcemia

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Introduction: hypercalcemia is associated with metabolic disorders, disturbance of bone metabolism, increased risk of death.

Objective: to study the prevalence of osteoporosis in postmenopausal women with hypercalcemia.

Materials and methods: we studied 31 postmenopausal women with hypercalcemia. Examination: total calcium, phosphorus, albumin, creatinine, PTG, vitamin D, DXA. The control group were 31 postmenopausal women without hypercalcemia, mean age 58,1±2,11 with physiological menopause (mean age of menopause starting 50±3,1 years).

Results: hypercalcemia was detected in 31 postmenopausal women, mean age 58±11,6 years, mean age of physiological menopause was 50,1±2,34 years. The were no differences in the age, vitamin D status, comorbid pathology in the postmenopausal women with hypercalcemia and control group. Hypercalcemia persisted from 1 to 5 years. Results of DXA in women with hypercalcemia: T-score L1-L4 total was -1,343±1,42, T-score right femur total was -1,017±0,79, T-score left femur total was -0,961±0,62. Results of DXA in women of control

group: T-score L1-L4 total was -0,814±1,01, T-score right femur total was 0,136±0,77, T-score left femur total was 0,139±0,64. Osteoporosis was founded in 13 cases in patients with hypercalcemia and in 4 cases in the group of control. Significant differences was detected in the prevalence of osteoporosis in postmenopausal women with hypercalcemia compared postmenopausal women without hypercalcemia (x2=6,56, p=0,0104).

Conclusion: The results of the study show increased risk of osteoporosis in postmenopausal women with hypercalcemia. The results may suggest the influence of hypercalcemia on the development of osteoporosis in postmenopausal women.

PO15

Acupuncture at Tiaokou (ST38) for shoulder adhesive capsulitis: what strengths does it have? A systematic review and meta-analysis of randomized controlled trials

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Background: Tiaokou (ST38) is commonly used as a crucial distal acupoint for treating Shoulder Adhesive Capsulitis (SAC) in TCM. However, there has been no systematic review summarizing the evidence concerning the effectiveness of acupuncture at Tiaokou.

Objective: To assess the current high quality evidence of the effects of acupuncture at Tiaokou for patients with SAC.

Methods: We searched eight electronic databases without language restrictions. All the literature was processed to identify RCTs comparing acupuncture at Tiaokou with other therapies (acupuncture at local shoulder acupoints or other therapies). Two reviewers extracted trials and collected outcome data independently. A meta-analysis was performed following a strict methodology.

Results: 20 RCTs involving 2014 participants met our inclusion criteria. The majority of the trials were determined to be of low quality. Both of acupuncture at Tiaokou individually or in combination with shoulder acupoints were superior to control methods in improving the percentage of clinical effectiveness (RR=1.27[1.05,1.53], P=0.01), (RR=1.13[1.03,1.23], P=0.007). Meanwhile, only acupuncture at Tiaokou in combination with shoulder acupoints was better than other therapies in alleviating Visual Analogue Scale (VAS) (WMD=-1.41[-1.54,-1.29](95%CI), P<0.00001), as well as in improving Constant-Murley Score (CMS) (WMD=11.45[3.25,19.64], P=0.006). There is not enough evidence to show that acupuncture at Tiaokou individually was more effective than other therapies in ameliorating VAS or CMS (WMD= -0.59[-1.20,0.01], P=0.06), (WMD=7.16 [-6.06,20.38], P=0.29).

Conclusion: Our systematic review finds encouraging evidence for the effectiveness of acupuncture at Tiaokou for SAC. Nonetheless, despite stringent methodological analyses, these results still need to be strengthened by additional RCTs of higher quality.

PO16

Role of osteoblast menin in bone metabolism: *ex vivo* studies of knockout mice

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In humans, mutations in the MEN1 tumour suppressor gene cause the Multiple Endocrine Neoplasia Type 1 disorder. Menin, the product of the MEN1 gene, is predominantly a nuclear protein that also facilitates cell proliferation and differentiation control. Our previous *in vivo* study illustrated the importance of menin for proper functioning of mature osteoblasts and maintenance of bone mass in adult mice. In the present study, we examined the *in vivo* role of menin at earlier stages of the osteoblast lineage through conditional knockout of the Men1 gene. This was implemented through the Cre-LoxP recombination system and Prx1-Cre; Men1f/f and Osx-Cre; Men1f/f mice represent knockout of the Men1 gene in the mesenchymal stem cell and the preosteoblast, respectively. Our results demonstrate impaired trabecular and cortical bone formation in the earlier menin knockout mice models. Mineralization and differentiation of the primary calvarial osteoblasts in the knockout mice were deficient relative to those of wild-type mice as assessed by Alizarin red and von Kossa staining. Gene expression profiling of RNA extracted from the primary calvarial osteoblast in Osx-Cre; Men1f/f mice revealed reduced osteoblast markers, increased proliferation markers and increased RANKL/OPG ratio that would favor osteoclastogenesis in the knockout animals. This is consistent with ongoing *in vivo* histomorphometric analysis that demonstrates an increase in osteoclast number and activity in the knockout animals. Osteoblast menin plays a crucial role in the development and maintenance of bone mass, and may serve as a potential gain-of-function therapeutic target for low bone mass disorders, such as osteoporosis.

PO17

Osteopenia in Marfan syndrome: role of osteocytes

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Bones have the capacity for self-repair and adaptation to changing mechanical loads. Osteocyte bone cells are thought to play a major role in sensing mechanical signals and coordinating bone formation and resorption. Thus, an altered osteocyte network morphology may directly affect bone's mechanoresponsiveness, with subsequent imbalances in bone formation and resorption contributing to low bone mass (osteopenia). Marfan syndrome (MFS) is a genetic disorder exhibiting osteopenia due to a genetic defect in fibrillin-1; however, the role of osteocytes in MFS has not been investigated to date. Here, we investigate the role of osteocytes in MFS using the *Fbn1*$^{C1039G/+}$ mouse model of MFS, in which heterozygous mice have a missense mutation in fibrillin-1. We hypothesize that osteocytes contribute to the osteopenic phenotype of MFS because of their abnormal lacuno-canalicular network, density and behavior. Consistent with the MFS phenotype, µCT and real-time qPCR evaluations show that *Fbn1*$^{C1039G/+}$ mice are osteopenic, exhibit long bone overgrowth and higher RANKL/OPG ratio compared to littermate control (LC). Furthermore, mechanical tests show trends towards lower elastic modulus and post-yield displacement in *Fbn1*$^{C1039G/+}$ compared to LC, which is consistent with lower mineralization in *Fbn1*$^{C1039G/+}$ mice. Osteocyte lacunar densities measured with µCT were similar, but real-time qPCR showed lower Wnt1 and Lef1 expression in *Fbn1*$^{C1039G/+}$ mice compared to LC. In conclusion, *Fbn1*$^{C1039G/+}$ mice exhibit the MFS skeletal phenotype and abnormal osteocyte signaling may contribute to altered mechanical properties in *Fbn1*$^{C1039G/+}$ mice. Future work will investigate whether osteocytes' mechanosensing ability can be exploited to reduce osteopenia in MFS.

P018

Therapeutic potential of osteoclast inhibitory fibrillin-1 fragments

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Marfan syndrome, due to mutations in fibrillin-1 gene, is a common type-I fibrillinopathy characterized by severe skeletal complications, including osteopenia and long bone overgrowth. How fibrillin-1 mutations lead to the skeletal problems is poorly understood. An N-terminal sub-fragment of fibrillin-1 (rF23) was identified as strong inhibitor of osteoclastogenesis *in vitro* and *in vivo* in healthy animals. To identify the potent osteoclast inhibitory sub-fragments of fibrillin-1, we produced several smaller fragments of rF23. The purified proteins were tested for their effect on osteoclastogenesis using primary osteoclasts. We observed reduced number and size of the differentiated osteoclasts. Next, we plan to examine if fibrillin-1 fragments exhibit sufficient anti-resorptive activity in a Marfan syndrome mouse model Fbn1mgR/mgR. To understand the baseline bone parameters, we analyzed bones of Fbn1mgR/mgR 4, 8, 12 and 16 weeks after birth. Compared to wild type littermates, young Fbn1 mgR/mgR mice show a trend of increased length of long bones, increased body lengths and decreased BMD which becomes significant at later time points. In osteoclastogenesis experiments, we found no significant difference in osteoclast number between the two groups. The expression of RANKL and OPG was increased in Fbn1mgR/mgR mice of both sexes, however RANKL/OPG ratio was affected differently - it was higher in Fbn1mgR/mgR males compared to wild type, but lower in females. In conclusion, we have identified smaller fragments of rF23 that exhibit osteoclast-inhibitory activity. Bone phenotype development in Fbn1mgR/mgR mice suggest that treatment can be started at 4 week old animals.

P019

Parathyroid cell transcription factor glial cells missing-2: Novel inactivating and activating mutations associated with hypoparathyroidism and hyperparathyroidism, respectively

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Context: Inactivating mutations in the parathyroid cell-specific transcription factor glial cells missing-2 (GCM2) cause hypoparathyroidism inherited in either a recessive or autosomal dominant fashion. Activating mutations in *GCM2* predispose individuals to familial isolated hyperparathyroidism.

Objective and subjects: To identify the causative mutations in two families with individuals presenting with hypoparathyroidism and twenty-four families with individuals presenting with hyperparathyroidism.

Methods: Leukocyte DNA of hypoparathyroid individuals underwent *CASR, PTH, GNA11* and *GCM2* gene mutation testing and that of hyperparathyroid individuals *MEN1, CDC73, CASR* and *GCM2* gene mutation testing. GCM2 variants identified were evaluated by *in vitro* functional analysis of GCM transcriptional activity.

Results: A homozygous novel c.199C>T; p.R67C *GCM2* variant was identified in affected members of both hypoparathyroid families. The mutant demonstrated markedly decreased activity in the *in vitro* functional assay relative to wild-type GCM2. Structural modeling indicated that C67 has lost the ability of R67 to interact with DNA. Heterozygous c.1144G>A;p.V382M (recurrent): c.1149C>G;p.I383M (novel): c.1156A>T;p.T386S (novel): or c.1181A>C;p.Y394S (recurrent) GCM2 variants that lie within a C-terminal conserved inhibitory domain were identified in affected individuals of four of the hyperparathyroid families. These mutants all demonstrated significantly greater *in vitro* functional activity than wild-type GCM2.

Conclusion: We have identified a novel inactivating mutation in the *GCM2* gene and provide evidence that it is causative of hypoparathyroidism. We have identified novel and recurrent activating mutations in the *GCM2* gene and provide evidence that they contribute to hyperparathyroidism.

P020

Mechanically-evoked ATP release is regulated by facilitated membrane resealing in murine osteoblasts

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ATP release is one of the first events to occur in response to mechanical stimulation of mammalian cells. However, there is ongoing debate about the dominant pathways involved in the release of this ubiquitous signalling molecule. We mechanically-stimulated compact bone-derived osteoblasts through direct membrane deformation or turbulent fluid shear. Using an ATP bioluminescence assay, we demonstrated that ATP was released in response to mechanical stimuli. We next examined the contribution of vesicular exocytosis to mechanically-induced ATP release. Using confocal microscopy, we confirmed that acidophilic dye, quinacrine, and fluorescent ATP analog, MANT-ATP, co-localize within vesicles. Mechanical stimulation of single osteoblast evoked rapid exocytosis of quinacrine-positive vesicles. Activation of protein kinase C (PKC) with phorbol-12-myristate 13-acetate (PMA) significantly potentiated vesicular exocytosis, while a broad-spectrum PKC inhibitor, bisindolylmaleimide II (BIS), significantly reduced exocytosis. Unexpectedly, increase in vesicular release coincided with a significant decrease in ATP released, and vice versa. Since PKC-mediated vesicular release has been implicated in membrane resealing, we examined membrane integrity in mechanically stimulated cells. By applying a membrane impermeable dye, Trypan blue (TB), before and 5 min after the mechanical stimulation, we have found that the membrane disrupted in cells immediately upon mechanical stimulation, however this effect was reversible within 5 min indicating active membrane resealing. PMA pre-treatment significantly reduced immediate TB uptake, while in cells treated with BIS, cells remained TB-permeable 5 min after mechanical stimulation. Our data supports a model in which mechanical stimuli routinely and transiently disrupt the cellular membrane, allowing ATP release into the extracellular space.