Aims: To estimate heritability of trabecular density (TD), cortical density (CD), trabecular thickness (TT) and cortical thickness (CT) at tibia and radius by high-resolution pQCT (HRpQCT) in mothers and their children, and to investigate the genetic correlations among these phenotypes at tibia and radius.

Methods: The study comprised 174 pairs from 161 families (161 mothers, mean age 52 years; 174 children, mean age 26 years). Cortical and trabecular bone microstructures at tibia and radius were measured by HRpQCT. SOLAR software was used to conduct quantitative genetic analyses with adjustment for covariates.

Results: In multivariable analysis, maternal tibial TD, CD, TT and CT were independently associated with all corresponding bone microstructures in the children (β=0.14 to 0.37, all P<0.05) after adjustment for age, sex, weight, height, smoking history and physical activity. Additive genetic factors explained 67%, 36%, 33% and 46% of the variation in TD, CD, TT and CT, respectively (adjusted P<0.05). Similar results were observed at the radius, but the heritability estimates were lower than that at tibia (range, 12%-57%). Genetic correlations for TD and TT between tibia and radius (rg=0.80 for TD; 0.86 for TT) were greater than that of CD and CT at tibia and radius (rg=0.47 for CD; 0.50 for CT).

Conclusion: This study suggests that genetic factors appear to have an important role in the development of peak bone microarchitecture and are partly shared for the tibia and radius, but these effects seem to be weaker for cortical bone as compared to trabecular bone.

Background: Abdominal aortic calcification (AAC) has prognostic value for adverse cardiovascular outcomes. Altered bone and mineral metabolism during ageing contributes to increased AAC. Loss of skeletal muscle mass also occurs with age and may contribute to bone demineralisation. We aimed to determine the association between low relative muscle mass and AAC in community-dwelling older Australians.

Methods: Cross-sectional study of 327 community-dwelling older adults (mean age and BMI 70.6±5.5yr and 28.2±5.2kg/m², respectively; 62% female). Appendicular lean mass (ALM) was determined by dual energy x-ray absorptiometry. AAC was determined by thoraco-lumbar radiography. Aortic calcification score (ACS; range 0-24) was calculated visually as the extent of calcification on the aortic walls between the L1-L4 vertebrae, and compared across sex specific tertiles of ALM normalised to body mass index (ALM/BMI).

Results: AAC prevalence was highest in patients in the bottom tertile of ALM/BMI [n=77(74.8%)] compared to middle [60(65.2%)] and upper [72(54.5%)] tertiles; (p=0.006). Median ACS was highest in the bottom tertile of ALM/BMI [4(0-12.5)] [median and (IQR)] compared to middle [2(0-5] and upper [1(0-4)] tertiles; (p=0.005). The upper tertile for ALM/BMI had decreased odds (β=-0.659; Odds=0.519; 95% confidence interval: 0.271-0.988; p=0.046) of having any AAC relative to the lower tertile independent of traditional risk factors of age, sex, body fat, smoking, hypertension, elevated total cholesterol and physical activity.

Conclusion: AAC is more prevalent and severe in community-dwelling older adults with low relative ALM. Prospective studies are required to determine whether low muscle mass predicts development and progression of aortic calcification.
Background: The association between lipids and bone mass in adult life is controversial and there is limited evidence in childhood. The aim of this study was to describe the association between cholesterol measured in childhood and young adult life and bone mineral density (BMD) in younger adults.

Methods: Subjects broadly representative of the Australian population (n=1431, female=52%, age=26-36) were selected from the Australian Schools Health and Fitness Survey of 1985. They underwent various measurements including leg strength, standing long jump, and physical work capacity (PWC170). Physical activity, smoking and alcohol history were recorded using questionnaires. Fasting cholesterol levels were assessed in childhood and 20 years later in adulthood. A single Sahara bone ultrasound densitometer was used to determine heel bone mass in adulthood.

Results: In multivariable analysis, childhood high-density lipoprotein (HDL) was positively (β: 63.86 mg/cm², 95%CI: 11.12,116.60) associated with BMD in adulthood. Adulthood total cholesterol (TCH) (β: -7.36 mg/cm², 95%CI: -14.51,-0.21) and low-density lipoprotein (LDL) (β: -10.90 mg/cm², 95% CI: -19.23,-2.58) were negatively associated with adult life BMD. Subjects who remained in the abnormal category of TCH from childhood to adulthood had the least bone mass compared to other category changes. These associations were independent of the potential confounders including physical activity.

Conclusions: HDL in childhood was beneficially associated with adulthood BMD. LDL and TCH in adulthood were detrimentally associated with BMD. The effect of childhood HDL was independent of the adulthood HDL levels, indicating that cholesterol may have long-term effects on bone mass from childhood to adulthood.

Figure 1: Mean bone mineral density (adjusted) for subjects in different categories (normal and high) of total cholesterol (TCH) change from childhood to adulthood
Background: The pathophysiology and natural history of normocalcemic hyperparathyroidism are not well established. High PTH is associated with cardiovascular disease and metabolic abnormalities, and in some individuals labelled with ‘normocalcemic hyperparathyroidism’, elevated PTH may reflect cardiometabolic disease rather than parathyroid dysfunction. We investigated relationships between PTH, cardiometabolic risk factors, and bone mineral density (BMD), focusing on individuals with high and high-normal PTH.

Methods: Healthy adult men (n=151) were studied. Anthropomorphic measurements, blood pressure, biochemistry, coronary artery calcium (CAC) scores and BMD/body composition were obtained. Relationships between variables were assessed.

Results: PTH correlated positively with diastolic blood pressure (DBP), BMI, fat mass, triglycerides, total cholesterol, LDL cholesterol and CAC score (r=0.19 to 0.26 and p=0.02 to 0.002). Men in the top PTH tertile (≥4.4 pg/mL, n=51) were more likely to have LDL cholesterol ≥3.5 mmol/L, DBP ≥85 mmHg, and CAC score >0 than men in lower tertiles. Amongst the top PTH tertile, men with the highest PTH levels (≥5.3 pg/mL, n=29) had higher DBP (85 versus 79 mmHg, p = 0.04) and higher triglycerides (1.60 versus 1.19 mmol/L, p = 0.01) than the remainder of men within this tertile (n=22).

PTH was not associated with history of fracture, baseline BMD, or change in BMD over two years.

Conclusions: In men recruited from the general population, high and high-normal PTH levels are reflective of an adverse cardiometabolic phenotype, and not indicative of skeletal health. ‘Normocalcemic hyperparathyroidism’ may not be an appropriate label for all individuals with high PTH in the absence of hypercalcemia or secondary hyperparathyroidism.

This study aimed to examine associations between objectively measured physical activity (PA) intensity and sedentary time with musculoskeletal health outcomes in middle-aged women. This cross-sectional analysis from a population-based sample of 309 women (aged 36-57 years) used linear regression to examine associations of accelerometer counts/hour and minutes/day of sedentary behaviour and light and moderate-to-vigorous PA (MVPA) (Actigraph GT1M accelerometer) with lumbar spine (LS) and femoral neck (FN) bone mineral density (BMD) (dual-energy X-ray absorptiometry), lower limb muscle strength (LMS), dynamic and static balance tests (timed up and go test (TUG), functional reach test (FRT), lateral reach test (LRT) and step test (ST)). Women had median PA of 20184 counts/hour. The median (minutes/day) for sedentary behaviour, light and MVPA were 535, 267 and 37, respectively. After adjustment for potential confounders, an increase of 10000 counts/hour was positively associated with FN BMD and LMS while negatively but beneficially associated with TUG (β (95%CI) = 0.014 (0.004-0.023); 3.7 (0.5-6.9); -0.13 (-0.21--0.04) for FN BMD, LMS and TUG, respectively). An increase in sedentary time of 100 minutes/day was negatively associated with FN BMD (β (95% CI) = -0.014 (-0.027-0.000)), though this was borderline statistically. There was a dose-response relationship between PA intensity, FN BMD, TUG and LMS, with the largest effect sizes observed for MVPA (β (95% CI) = 0.0051 (0.0008-0.0095); -0.040 (-0.067--0.013); 1.49 (0.44-2.53) for FN BMD, TUG and LMS, respectively).

Greater PA was associated with better musculoskeletal health in middle-aged women, and more MVPA and less sedentary time are preferable.
PLENARY POSTER – P6
KNEE OSTEOARTHRITIS: BONE MARROW LESIONS DETECTED BY SPECIFIC MRI SEQUENCES ASSOCIATE WITH SEVERITY OF OSTEOCHONDRAL DEGENERATION
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1Discipline of Orthopaedics Trauma, the University of Adelaide, 2Department of Epidemiology Preventive Medicine, Monash University, 3Anatomical Pathology, Sa Pathology, 4Bone and Joint Research Laboratory, Sa Pathology, 5Adelaide Microscopy, the University of Adelaide, 6Department of Radiology, Royal Adelaide Hospital, 7Department of Orthopaedics, Repatriation General Hospital

Aims of the study: MRI-identified bone marrow lesions (BMLs) are subchondral bone abnormalities that closely associate with joint pain and structural degeneration in knee osteoarthritis (OA). Tissue changes within BMLs and their influence on OA progression remain unclear. Thus, this study aimed to provide comprehensive tissue-level characterisation of BMLs and to investigate potential differences using specific MRI sequences.

Methods: Tibial plateaus (TP) were obtained from 60 patients (29-females, 31-males), aged 51-87 years, undergoing knee arthroplasty for OA. T1 and PDFS-weighted MRI scans were performed and images used for cartilage and BML volume measurement. Bone microstructure was assessed by micro-CT. Histopathology of the whole osteochondral unit (OCU) enabled OARSI grading and routine pathologist assessment. Static bone turnover indices were quantified.

Results: BMLs were detected in 74% TP; the remainder formed No-BML group. BML-1 (PDFS) represented 44%; BML-2 (PDFS+T1) represented 30%. BML presence indicated moderate to advanced OCU degeneration (summarised Table 1). The most significant changes where within BML-2; reduced cartilage volume (p=0.008), higher OARSI score (p=0.004), thicker subchondral plate (p=0.002), increased trabecular bone volume and plate-like structure (p=0.0004), increased osteoid volume and thickness, more bone marrow oedema, fibrosis (p=0.002), necrosis (p=0.01) and fibrovascular cysts (p=0.04), compared to No-BML. For most measures, BML-1 was intermediate between No-BML and BML-2.

Conclusion: BMLs detected by specific MRI sequences are characterised by different degrees of osteochondral degeneration. These data suggest that BMLs may be potential MRI biomarkers for identification of individuals at high risk of progressive OA and/or for development and monitoring of new therapies.

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<th>Table 1</th>
<th>BML-1 (PDFS only)</th>
<th>BML-2 (PDFS + T1)</th>
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<td>Cartilage</td>
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<td></td>
<td>OARSI histology score</td>
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<td>Bone marrow pathology</td>
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<td></td>
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<td>Osteoid volume and thickness</td>
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No-BML vs. BML-1 and BML-2.
DELETION OF PROTEASE ACTIVATED-RECEPTOR 2 (PAR2) IMPROVES BONE AND MUSCLE PATHOLOGY IN THE MDX MOUSE
Taghaviesfandouni Neda1, Sanaei Reza1, Samuel Chrishan S2, Pagel Charles N1, MacKie Eleanor J1
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Duchenne muscular dystrophy (DMD) is associated with osteoporosis, and dystrophic mdx mice show reduced bone mass characterised by decreased mineral apposition along with elevated bone resorption. To investigate a potential role of the G-protein-coupled receptor protease-activated receptor 2 (PAR2) in the muscle and bone pathology associated with DMD, we established a colony of PAR2-null-mdx mice. Limb and diaphragm muscles, tibiae and serum of PAR2-null-mdx and littermate mdx mice from just after the onset of muscle pathology (4 weeks) until 20 weeks of age were examined. By 8 weeks, serum creatine kinase activity was lower in PAR2-null-mdx mice compared to mdx, and continued to drop in PAR2-null-mdx mice over time. From 8 weeks, in all muscles examined histologically, the area of active inflammation and the number of damaged fibres were lower in PAR2-null-mdx mice compared to mdx mice. Hydroxyproline content in the diaphragm (indicative of fibrosis) was significantly lower in PAR2-null-mdx than in mdx mice from 12 weeks onwards. PAR2-null-mdx mice were significantly stronger and more fatigue resistant from 8 weeks of age onwards. Micro-CT evaluation of the tibial metaphysis at 20 weeks of age showed that BV/TV and trabecular number were higher and trabecular separation lower in PAR2-null-mdx than in mdx mice. The IL6 and RANKL concentration, and the RANKL/OPG ratio in serum were lower in PAR2-null-mdx mice compared to mdx mice. These results suggest that PAR2 activation contributes to muscle and bone pathology in dystrophin deficient mice and that antagonising PAR2 may help ameliorate the effects of dystrophin deficiency.
Genes are a major determinant of bone mass yet heritability studies have identified a fraction of those that influence bone. Transcriptome sequencing, a powerful tool for unbiased genetic discovery, has been scarcely applied to bone tissue, and its capacity to examine the regulatory long non-coding RNA (lncRNA) architecture of bone has not been explored.

Tibiae, femora and humeri were isolated from 16 week, male mice (n=8), the marrow removed, and total-RNA sequenced to ~30 million reads/sample. Contra-lateral limbs were isolated for microCT and histological analysis to confirm reproducibility of the processing procedure. A bespoke analytical pipeline was constructed to define an osteocyte signature - genes enriched in cleaned bone samples and actively expressed in all sources of osteocytes.

A 1428-gene osteocyte signature was defined, encompassing the established osteocyte genes Sost, Dmp1 and Mepe, as well as 116 lncRNAs. 84% of the signature was not annotated with bone-related terms in the GO ontological database, suggesting genes novel to osteocyte biology are being detected in this high-confidence signature. These genes include Fat1, a transmembrane receptor implicated in growth signaling, Magi2, a scaffold protein essential for synapse development and Vldlr, a receptor critical in lipid metabolism involved in neurogenesis. Comparison of bone types identified 5 transcription factors, Pitx1, Hoxc8, Hoxc9, Hoxc10 and Hoxc11, actively expressed in tibiae and femora, but not humeri.

This profile of the osteocyte transcriptome has defined coding and non-coding genes that are novel in bone and identified genes restricted to bone types, providing new insights into genes that influence bone.

Osteoporosis occurs due to impairment of the highly orchestrated mechanism of bone homeostasis, resulting in brittle bones that are more susceptible to fracture. ENU-induced mutant mice generated through a collaboration with the Australian Phenomics Facility (ANU) were screened for bone phenotypes to identify key molecules that regulate bone homeostasis. We discovered a mouse line which carries a S24P mutation in the BH4 domain of the Bcl2 gene exhibiting osteoporosis. Previous studies have shown that global knockout of Bcl2 in mice results in reductions in bone formation, however, the specific role of Bcl2 in bone remains unclear. This project aimed to characterise the phenotype of the mutant mice and correlate variation in the BCL2 gene in humans with BMD in human populations. Bone structure and bone histomorphometry confirmed the osteoporotic phenotype. In vitro osteoclast and osteoblast cultures revealed no clear changes in differentiation, but indicated potential changes in osteoclast function due to alterations in calcium regulation. Analysis of common variation in the human BCL2 gene (+/- 10 Kb) using the GEFOS (GEnetic Factors for Osteoporosis) consortium dataset showed significant association of variants resulting in upregulation of BCL2 gene expression with reduced femoral BMD. We have identified mutations in the BCL2 gene that have direct effects on bone cell function and BMD in both mice and humans. Our analysis of the human BCL2 gene suggests that enhanced BCL2 gene expression is associated with reduced BMD. Further study is required to understand how changes in Bcl2 activity impact specific cellular functions in bone cells.
PLENARY POSTER – P10
A ROLE OF FKB12 IN ACTIVATION OF MUTANT ALK2 RESPONSIBLE FOR FIBRODYSPLASIA OSSIFICANS PROGRESSIVA (FOP)
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Aims: Fibrodysplasia ossificans progressiva (FOP) is a rare hereditary disorder characterized by progressive heterotopic ossification (HO) in skeletal muscle. FOP is caused by gain-of-function-mutations of ALK2, which is a type I BMP receptor. It has been suggested that those mutant ALK2 were reduced in a binding affinity to FK506-binding protein-12 (FKBP12), which is a repressor of BMP receptors. In the present study, we examined the role of FKBP12 in the activation mechanism of ALK2 by analyzing biochemical characteristics of a mutant ALK2 carrying a mutation at the FKBP12-binding domain, delP197-F198_insL (PF197-198L).

Methods: We examined the effect of FKBP12 on BMP signaling induced by an over-expression of ALK2 in C2C12 cells.

Results and Conclusion: Co-expression of FKBP12 dose-dependently reduced a BMP activity induced by ALK2(R206H), a recurrent mutation in patients with typical FOP. Similar reduction by FKBP12 was observed in atypical mutations except ALK2(PF197-198L). Co-IP experiments revealed that ALK2(R206H) bound to FKBP12, but ALK2(PF197-198L) did not. However, the patient carrying ALK2(PF197-198L) was not severe as patients carrying ALK2(R206H). Recently, we have reported that mutant ALK2 responsible for FOP were activated through phosphorylation by BMP type II receptors. In the presence of BMP type II receptors, FKBP12 did not inhibit the BMP signaling of ALK2(PF197-198L) nor ALK2(R206H). Taken together, our findings suggest that HO in FOP is induced by a cooperation of mutant ALK2 and BMP type II receptors. Although FKBP12 suppresses ALK2 in vitro, such repression may be cancelled by BMP type II receptors during HO.

PLENARY POSTER – P11
SODIUM SELENATE TREATMENT AMELIORATES BONE LOSS IN THE FEMORAL DISTAL METAPHYSIS FOLLOWING TRAUMATIC BRAIN INJURY IN RATS
Brady Rhys¹, Shulz Sandy², Sun Mujun², Romano Tania¹, Wright David³, O’Brien Terence⁴, Grills Brian¹, Stuart McDonald¹
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Aim: Sodium selenate treatment attenuates brain damage and improves behavioural outcomes in rats subjected to traumatic brain injury (TBI)¹. Recent work from our laboratory demonstrates that TBI caused region-specific bone loss in rats². Little is known on how attenuating brain injury affects bone loss. Here we investigated the effect of sodium selenate treatment on the quantity and quality of bone within the femur of rats subjected to TBI.

Methods: Rats were randomly assigned into either sham or TBI groups and administered either PBS or sodium selenate via min-osmotic pumps for 12 weeks and then killed. Femora were analysed both histomorphometrically and by peripheral quantitative computed tomography (pQCT). Biomechanical properties of the mid-shaft of femora were measured by three-point bending. Results: Femora from both selenate-treated groups were shorter in length compared to both vehicle-treated groups (p < 0.001). Distal metaphyseal trabecular bone volume fraction of TBI-selenate rats was higher than TBI-vehicle rats (p < 0.01). pQCT demonstrated that cortical thickness was decreased at the distal metaphyseal region in TBI rats compared to shams (p < 0.05). Furthermore, selenate treatment to TBI animals offset this reduction in cortical thickness in this region (p < 0.05). There were no differences in the mechanical properties at the mid-diaphysis between the four groups.

Conclusion: These findings indicate that selenate treatment disrupts long bone growth in young rats. However, attenuation of the brain injury by selenate treatment reduced the degree of metaphyseal trabecular bone loss post-TBI, which suggests a possible correlation between the extent of neurodegeneration and the severity of bone loss.

PLENARY POSTER – P12
IGF-1 PROTECTS GC INDUCED CONNEXIN43 DEGRADATION VIA ATTENUATING AUTOPHAGY IN OSTEOCYTES
Gao Junjie, Cheng Tak Sum, Pavlos Nathan, Zheng Minghao
The University of Western Australia

Therapeutic effects of glucocorticoid (GC) administration are accompanied by severe bone loss. Osteocytes, which comprise over 90% of the bone cell population and have a well-developed dendrite network for cell-cell communication, are highly susceptible to the adverse events of GC administration. IGF-1 mainly produced by osteocytes in bone is a key anabolic regulator for bone formation and developmental bone growth. Here we proposed that GC may induce osteocyte autophagy and diminish cell-cell communication between osteocytes. IGF-1 may be able to attenuate the adverse events caused by GC in osteocytes. In an attempt to identify genes involved in the effect of GC induced osteocyte autophagy, we conducted micro-array analysis on mouse primary osteocytes. We identified a cluster of key autophagy regulator genes and downstream factors for cell-cell communications. We showed that GC significantly induces autophagy in primary osteocytes from LC3-GFP mice. Furthermore, we observed pronounced morphologic changes and significant reduction and shortening of dendritic processes in primary osteocytes isolated from Dmp1-GFP mice following GC treatment. Osteocytic plasma membrane/dendrite associated connexion 43 (Cx43) was internalized into autophagosome/autolysosomes after the induction of autophagy. Degradation of Cx43 was attenuated following lysosomal inhibition with chloroquine or siRNA knockdown of autophagic related proteins, including Atg4b or Atg5. Interestingly, while GC reduces IGF-1 production in osteocytes, IGF-1 re-activates Akt-mTORC1 signalling cascade to attenuated GC induced autophagy and Cx43 degradation. Together, we demonstrated that GC severely suppressed osteocyte cell-cell communication via autophagy and IGF-1 protects against GC induced Cx43 degradation and autophagy via re-activation of Akt-mTORC1 signaling pathway.

PLENARY POSTER – P13
CHONDROCYTE ER STRESS AS A PATHOGENIC FACTOR IN OSTEOARTHRITIS?
Kung Louise1, Mullan Lorna1, Briggs Michael2, Boot-Handford Raymond1
1The University of Manchester, 2Newcastle University

Introduction: Osteoarthritis (OA) is a degenerative joint disease characterised by the progressive breakdown of articular cartilage. There is some evidence that increased ER stress is a feature of OA. Here, we investigated the relationship between the development of OA and ER stress in mice and whether increased ER stress in articular chondrocytes plays a pathological role in the OA disease process.

Methods: OA was induced in 10-week-old male wild type (+/+) and ColII Tg
tg
(c/c) mice by DMM surgery of the right knee. c/c mice have increased ER stress that has been induced in articular chondrocytes through the collagen II promoter driven-expression of ER stress-inducing Tg
tg
. Knee joints were dissected, fixed in 4% PFA, decalcified, paraffin embedded, sectioned and examined histologically. RNA-seq was performed on laser-micro-dissected RNA from cartilage of +/- and c/c mice.

Results: Increased Col2a1 and BiP expression was apparent from 2 weeks post DMM, in a focal region immediately adjacent to the OA lesions in +/- and c/c mice. c/c mice exhibited significantly reduced OA severity accompanied by a delay in apoptosis and increased BiP protein compared with +/- mice at 2 weeks post DMM. RNA-seq analysis revealed Xbp1-regulated networks to be significantly activated in c/c mice at 2 weeks post DMM.

Conclusion: Here, the increased ER stress in c/c mice appears to be playing a chondro-protective role against surgically-induced OA. Studies exploring the role of Xbp1 and the mechanism by which it may inhibit OA progression are currently being investigated.
PLENARY POSTER – P14
VERSICAN (V1) ALTERS THE ADHESIVE AND MIGRATORY PROPERTIES OF MACROPHAGES
Kaczmarek Adrian, Dunning Kylie, Rodrãguez-Baena Javier, Ricciardelli Carmila, Russell Darryl
University of Adelaide

Growing evidence implicates secreted proteoglycans as regulators of innate immune trafficking and signalling during inflammatory situations. Versican is one such proteoglycan, whose abundance within the extracellular matrix has been associated with an increase in innate immune cell infiltration and activation.

Interestingly, versican is also upregulated within the extracellular matrix of tumours, and has been postulated to be central in the regulation of the immune profiles of cancers. Adamts1 is the dominant protease of versican, and we have previously demonstrated that Adamts1-/- tumours show reduced cleaved versican; reduced tumour growth and an accompanying significant increase in CD45+ leukocyte infiltration. These results led us to propose that versican regulates immune cell infiltration during inflammation. To investigate these observations further, we have developed a method for the production of pure endotoxin free full-length recombinant V1-versican, and have explored its role in regulating the behaviour of macrophages.

In the current study, we have demonstrated that intact V1-versican is able to directly regulate various aspects of macrophage physiology. Treatment of RAW264.7 macrophage-like cells with recombinant full-length versican induced a significant dose-dependent decrease in cell adhesion to fibronectin (P<0.0001), with 3D-confocal imaging demonstrating a notable change in cell morphology (changes to lamellipodia, filopodia and focal adhesion occurrence), and up to a 75% decrease in associated cell spread (P<0.001). Interestingly, treatment with recombinant versican also induced a significant increase in cell migration (P<0.001) with confocal live-cell imaging suggesting a change in migratory rate, whilst still maintaining their decreased adhesive capacity.

Ongoing studies continue to investigate molecular pathways by which intact V1-versican is able to induce these changes in macrophage physiology, and how other cells of myeloid lineages may utilise versican during tumour infiltration.

PLENARY POSTER – P15
BLOCKING AGGREGAN INTRERGLOBULAR DOMAIN CLEAVAGE BY ADAMTS DOES NOT PROTECT AGAINST ALLODYNIA ASSOCIATED WITH OSTEOARTHRITIS IN MICE.
Jackson Miriam1, Zaki Sanaa1, Ravi Varshini1, Moradi Babak2, Smith Susan1, Fosang Amanda3, Little Christopher1
1Kolling Institute, University of Sydney, Raymond Purves Bone and Joint Research Laboratories, Rnrah, 2Orthopaedic Clinic, University of Heidelberg, Germany, 3University of Melbourne, Murdoch Childrens Research Institute, Parkville, Victoria

Introduction: Pain is the foremost clinical feature of osteoarthritis (OA), but the mechanisms controlling joint pain are poorly defined. ADAMTS5-null mice have reduced cartilage damage and mechanical allodynia following destabilization of the medial meniscus (DMM) to induce OA. To further investigate the role of ADAMTS-driven aggrecanolysis in OA, we characterised pain behaviour in Jaffa mice that have aggrecan resistant to ADAMTS cleavage and chondro-protection post-DMM.

Methods: Ten-week-old male Jaffa and C57BL6 wild-type (WT) mice underwent DMM. At intervals out to 16 weeks post-DMM animals were sacrificed and pathology scored histologically. In mice sacrificed at 16 weeks mechanical allodynia was measured prior to and at intervals post-DMM.

Results: Compared with WT, Jaffa had reduced cartilage proteoglycan loss and degradation up to 8 weeks, and subchondral bone sclerosis at 4 weeks (p<0.05), but chondro-protection was no longer evident after 12 weeks. There was no difference between genotypes in withdrawal threshold pre-operatively. Jaffa had more allodynia than WT at 2 weeks, but there was no difference compared with WT thereafter.

Discussion: These results confirm chondro-protection in Jaffa mice after DMM. As the disease in Jaffa eventually manifests to the same degree as WT, long-term protection in ADAMTS5-null mice may be associated with reduced cleavage of proteins other than aggrecan. The lack of resistance to mechanical allodynia in Jaffa suggests it is not reduced aggrecanolysis, cartilage degradation or early subchondral bone remodelling that regulates pain in this OA model. There may be direct effects of ADAMTS5 ablation on pain perception and sensitization in OA.
PLENARY POSTER – P16
THE ROLE OF MICRORNA-10B WITHIN EGF MEDIATED EMT
Wesccott David, Gasch Christin, Mellick Albert
Deakin University, School of Medicine

MicroRNAs are small non-coding RNAs that play a role in the expression of key genes, and have been implicated within breast cancer and the progression through EMT. Our lab has previously observed heterogeneous expression of miR-10b in circulating tumour cells from breast cancer patients, which may be indicative of malignancy. The aims of the study were to analyze the expression of miR-10b during the process of EMT and determine its regulatory role within it. PMC42-LA cell lines were used to determine the levels of miR-10b during the process of EMT. Using anti-miRNA and pre-miRNA-10b to treat the cells, changes in key EMT markers were seen. Results were gathered using qPCR, ISH and wound healing assays. ISH revealed higher expression levels of Vimentin corresponded with increased levels of miR-10b within EGF treated cells. qPCR confirmed the increase expression of miR-10b, however relative expression was low. Additionally qPCR showed little changes in the relative expression of key EMT markers such as E-Cadherin and Vimentin between anit-miR and pre-miR treated WT cells, however a greater increase in expression of Vimentin and SNAIL after treatment of EGF in cells treated with pre-miR-10b was observed. Cell motility was greatly increased in cells treated with EGF, and differences were seen between cells that were treated with pre- and anti-miRs, with slight differences seen between WT pre- or anti-miR-10b cells. The data suggests that miR-10b is one regulator of the many pathways of EGF mediated EMT, and plays a role in enhancing the effects of EMT.

PLENARY POSTER – P17
ADVANCED LIVE IMAGING TO MONITOR ENZYMATIC TARGETING OF TUMOUR-STROMAL FEEDBACK IN PANCREATIC CANCER
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Cancer initiation and development occur in a complex three-dimensional microenvironment with reciprocal feedback from the surrounding host tissue. In pancreatic cancer, an abundant deposition of fibrotic extracellular matrix (ECM) occurs and contributes to cancer initiation, progression, invasion and chemoresistance. In particular, increased deposition of hyaluronic acid (HA) has been observed in this disease, however the pathological significance of HA rheological and signalling properties remains misunderstood. In this study, we investigated how targeting HA with PEGPH20 in pancreatic cancer models derived from genetically engineered mouse models and stratified patient samples may affect cell invasion and chemotherapy response. We use 3D organotypic assays coupled with advanced live FRET imaging to optimize treatment with PEGPH20. 3D organotypic assays can recapitulate the biochemical and physical properties of the ECM in vitro, while FRET imaging enables us to dynamically monitor molecular events at the cellular and subcellular level. Analysis of stromal HA and ECM texture in a human pancreatic tissue microarray has been conducted to identify patients with “high HA” vs “low HA” and monitoring of HA targeting in these samples is achieved using patient-specific organotypic assays. We wish to assess whether priming of pancreatic tumour with PEGPH20 can reestablish normal tissue homeostasis, affect cell invasion and improve chemotherapy. We propose that, in the future, HA deposition could be used as a biomarker to identify patients that would specifically from ECM targeting and that FRET imaging could be a useful preclinical tool in animal models prior to patients translation.
PLENARY POSTER – P18
CCL5/CCR5 AXIS IN BREAST CANCER ANGIOGENESIS.
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1Deakin University, 2Griffith University

The chemokine ligand RANTES/CCL5 and its cognate receptor (CCR5) have recently been implicated in breast cancer neovascularization and spread. Bone marrow (BM)-derived endothelial progenitor cells (EPCs) regulate the angiogenic switch via paracrine secretion of pro-angiogenic growth factors, and by direct luminal incorporation into sprouting nascent vessels. In support of a role for CCR5 in mature tumor vasculature, we demonstrate that CCR5 is up-regulated in endothelial cells in response to tumor-conditioned medium by performing transwell assay. We knocked out CCR5 with CCR5 sRNA and used tumor condition media as attractant. It resulted in decreased migration and showed that specific suppression of CCL5/CCR5 signaling in endothelial cells leads to significant angiogenic defects. Our further research revealed significant changes in the activation states of key members of PI3K/Akt signal pathway on treating endothelial cells with CCL5 and Maraviroc (CCR5 inhibitor). Lysates were subjected to activation analysis using the PathScan Akt Signaling Antibody Array followed by Western blot validation. Key member of this pathway GSK3 is upregulated in CCL5 treated cells and down regulated in Maraviroc treated cells. GSK-3 has been implicated in angiogenesis. Therefore it suggests that tumour CCL5 is promoting neoangiogenesis by enhancing recruitment and differentiation of endothelial cells through activation of key members of Akt pathway. The activity of GSK can be blocked by Thiazolidinedones (TZDs), currently used clinically to treat diabetes and which have anticancer properties. CCL5/CCR5 axis is important in tumor angiogenesis driven by pre-existing vasculature, and directly targeting CCR5 expressing vasculature may constitute a novel strategy for inhibiting tumor angiogenesis in breast cancer.

PLENARY POSTER – P19
VITAMIN D METABOLITES PROTECT AGAINST UV-INDUCED SKIN CANCER AND PHOTOAGING
McCarthy Bianca, Coles Lucy, Tongkao-on Wannit, Lee Seung Ho, Painter Nicole, Rybchyn Mark, Reeve Vivienne, Dixon Katie, Mason Rebecca
University of Sydney

There is some evidence that for young people, messages to use sun protection to reduce premature aging of the skin may be a powerful strategy to improve sun-protection behaviour and reduce skin cancer risk. In light of this, it would be helpful to know whether compounds other than sunscreen filters can reduce photocarcinogenesis and photoaging. The active vitamin D metabolite, 1,25-dihydroxyvitamin D₃, has already shown to protect against DNA damage, immune suppression, and photocarcinogenesis. The role of this vitamin D metabolite and other similar compounds in the protection against photoaging, however, has not yet been determined. In this study, albino hairless mice were irradiated with UV and then topically treated with a vitamin D metabolite or a vehicle control for 10 weeks. After a further 30 weeks, dorsal skin was examined for markers for photoaging: epidermal thickness, and elastin quality and quantity. Epidermal thickness was quantified by skin sections stained by haematoxylin and eosin, while elastin was quantified by sections stained by aldehyde-fuchsin. Vitamin D metabolites were found to inhibit UV-induced epidermal thickening (p<0.05) when compared to age-matched mice that had not been exposed to UV. Similarly, these compounds inhibited UV-induced increase in elastin (p<0.01) and elastin disorganisation (p<0.01). These results correlate with the ability of the same compounds to protect against DNA damage in the same model. In the future, topical application of vitamin D metabolites may be used as an adjunct strategy to protect against both photoaging and skin cancer.
Mucopolysaccharidosis (MPS) type IIIA results from a deficiency in the enzyme sulphamidase, leading to the lysosomal accumulation of the glycosaminoglycan (GAG) heparan sulphate (HS) within the central nervous system. HS is vital to many signalling pathways involved in neurogenesis, and the ability of HS to bind to the signalling molecules in these pathways is determined by the sulphation patterns along the length of the chain. HS from MPS IIIA displays altered sulphation patterns compared to normal HS, which may interfere with neurogenesis. In this study we investigated the neurogenic capacity of mesenchymal stem cells (MSC) isolated from MPS IIIA and normal bone.

Normal and MPS IIIA MSCs were isolated from compact bone and expanded in culture. Normal MSCs proliferate rapidly and differentiate down the neurogenic lineage as evidenced by the expression of nestin, β-tubulin and neurofilament-M in response to neurogenic differentiation medium. MPS IIIA MSCs, however, failed to proliferate and cell number decreased with time. Supplementation of exogenous sulphamidase to the cell media restored the proliferative capacity of MPS IIIA MSCs. These results suggest that the lysosomal turnover of HS chains is crucial to MSC proliferation and neurogenesis.
Poster Presentations - Clinical

P21
RACIAL DIMORPHISM IN BONE MICROSTRUCTURE
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Chinese have lower non-vertebral fracture rates than Caucasians, but vertebral fracture prevalence does not differ. To quantify the microstructural basis of these racial differences we studied 75 Chinese and 168 Caucasian premenopausal, and 49 Chinese and 122 Caucasians postmenopausal women in Melbourne. The distal radius was scanned using HR-pQCT (Scanco, XtremeCT) and morphology was quantified using StrAxi1.0. Trabecular plate (p) and rod (r) bone volume fractions (BV/TV) were analysed by Individual Trabeculae Segmentation (ITS). Bone strength was estimated by micro-finite element analysis (µFEA).

Premenopausal Chinese assembled a 13.9% smaller total cross sectional area (CSA), an 18.1% smaller medullary area. Chinese had a lower compact-appearing cortex (CC) and outer transitional zone (OTZ) porosity than Caucasians (32.7±4.0 vs. 35.7±4.3%; 37.1±3.5 vs. 39.1±3.5%; respectively, p<0.001). Trabecular pBV/TV was 25.6% higher and rBV/TV was 7.5% lower. There were no racial differences in bone strength. Chinese had a higher matrix mineral density (67.2±1.7 vs. 66.1±1.6%, p<0.001).

Postmenopausal Chinese and Caucasians had similar cortical thickness in absolute terms, but Chinese had thicker cortices relative to their smaller total CSA. Total cortex, CC and OTZ porosity did not differ by race. Nor was pBV/TV different but the rBV/TV was 20.1% lower in Chinese. The loss of trabecular plates contributed to 11.5% lower failure load in Chinese. We infer that Chinese assemble a more robust macrostructure which is maintained after menopause but advantages of lower porosity and higher trabecular plate numbers appear to be lost suggesting Chinese may lose relatively more bone from their smaller skeleton during menopause.

P22
VITAMIN D RECEPTOR PROTEIN EXPRESSION IN BREAST CANCER
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1Bone Research Program, Anzac Research Institute, 2Bone Research Program, Anzac Research Institute; the University of Sydney

Our prior animal research demonstrates that loss of the vitamin D receptor (VDR) in breast cancer cells is associated with more aggressive tumour behaviour and promotes cancer metastasis to bone. In the current study we investigated whether VDR protein expression in surgical breast cancer specimens correlates with disease progression and patient outcomes.

Methods: Tissue microarrays of 171 patients with breast cancer were obtained from the Australian Breast Cancer Tissue Bank, together with data on hormone receptor status, cancer grading and clinical outcomes (≥5-year follow-up). Expression of VDR, CYP24 and E-cadherin was assessed by immunohistochemistry using a validated scoring system.

Results: Of the 171 specimens, n=29 were histologically well differentiated (grade 1) while n=66 and n=76 were intermediate (grade 2) or poorly differentiated (grade 3). Compared to grade 1 and 2 cancers, VDR expression was significantly lower in poorly differentiated tumours (p<0.05). Consistent with these results, VDR expression levels were significantly lower in cancers with poor clinical prognosis (Her2, n=8; triple negative breast cancers, n=20) than in those with better outcomes (luminal A, n=112; luminal B, n=17) (p<0.05). Accordingly, the primary cancers of all 23 patients with distant metastasis or recurrences showed low VDR expression patterns. Of note, CYP24 and E-cadherin protein expression were positively associated with VDR expression.

Conclusion: Low VDR, CYP24 and E-cadherin expression are associated with poor cancer cell differentiation and a less favourable clinical prognosis. These clinical results confirm experimental work suggesting that the VDR plays an important role in cancer progression and dissemination.
Osteonecrosis of the jaw (ONJ) is a rare but serious adverse event of some antiresorptive therapies, and invasive oral procedures and events (OPEs) are an important risk factor. The incidence of positively adjudicated ONJ in the antiresorptive denosumab (DMAB) clinical program is rare (between ≥1 and <10 per 10,000). This study assessed the occurrence of invasive OPEs through Year 5 of the ongoing, 10-year FREEDOM Extension (EXT) trial. In FREEDOM, women were randomized to receive DMAb 60 mg SC or placebo Q6M for 3 years. In FREEDOM EXT, Women in the EXT long-term group (N = 2343) received DMAb in FREEDOM and EXT, and women in the EXT cross-over group (N = 2207) received placebo in FREEDOM and DMAb in EXT. Over 5 years of the EXT, 42.4% of participating women reported an invasive OPE; the incidence of the 5 individual OPEs reported was similar between groups (Table 1). ONJ incidence was 0.4% (7/1500 subjects) in women reporting invasive OPEs and 0.05% (1/2036 subjects) in women reporting no invasive OPEs. During the EXT (Years 1–5), the exposure-adjusted incidence of ONJ was 4.2 per 10,000 patient years. Of the 8 ONJ cases, 6 have resolved, 1 is ongoing and continues to be followed, and the final outcome of 1 is unknown, as consent was withdrawn. While invasive OPEs were common in this group of DMAb-treated women with postmenopausal osteoporosis, ONJ incidence was low. Invasive OPEs will continue to be queried prospectively in the EXT to characterize the long-term background rate.

**Table 1**

<table>
<thead>
<tr>
<th>FREEDOM Extension Years 1–5</th>
<th>Long-term (N = 1827)</th>
<th>Cross-over (N = 1709)</th>
<th>All (N = 3536)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years, mean (SD)</td>
<td>74.4 (4.7)</td>
<td>74.2 (4.8)</td>
<td>74.3 (4.8)</td>
</tr>
<tr>
<td>Any invasive oral procedure or event</td>
<td>763 (41.8)</td>
<td>737 (43.1)</td>
<td>1500 (42.4)</td>
</tr>
<tr>
<td>Scaling or root planing</td>
<td>500 (27.4)</td>
<td>481 (28.1)</td>
<td>981 (27.7)</td>
</tr>
<tr>
<td>Tooth extraction</td>
<td>387 (21.2)</td>
<td>354 (20.7)</td>
<td>741 (21.0)</td>
</tr>
<tr>
<td>Dental implant</td>
<td>88 (4.8)</td>
<td>79 (4.6)</td>
<td>167 (4.7)</td>
</tr>
<tr>
<td>Natural tooth loss</td>
<td>59 (3.2)</td>
<td>57 (3.3)</td>
<td>116 (3.3)</td>
</tr>
<tr>
<td>Jaw surgery</td>
<td>11 (0.6)</td>
<td>9 (0.5)</td>
<td>20 (0.6)</td>
</tr>
</tbody>
</table>

N = Number of subjects who received ≥ 1 dose of investigational product in FREEDOM Extension and responded to ≥ 1 oral event questionnaire related to FREEDOM Extension. n = Number of subjects with ≥ 1 invasive oral event.
P24
RELATIONSHIP BETWEEN PORE SHAPE AND POROSITY
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INTRODUCTION. Porosity is a major determinant of bone fragility. Pores may weaken the bone by either decreasing the amount of load bearing area or by acting as stress concentrator. The latter is due to pores becoming irregular in shape. We hypothesized the higher the porosity, the more irregular the pores contributing to the porosity.

MATERIALS AND METHODS. We studied 20 specimens of women aged 29 to 87 assessed using scanning electron microscopy. Pore shape was measured as deviation from circularity. Pore size and porosity were measured.

RESULTS. In the compact-cortex, pores of diameter ranging 25 to 48µm were circular. Above 48 µm diameter pores became increasing elliptical [r=0.245; p<0.0001]. Similar findings were made in the transitional zone, the larger pores the irregular their shape [ r=0.259; p<0.0001]. In both cortical compartments, the higher the porosity, the more irregular the shape of pores contributing to the porosity, respectively r =0.395; p<0.0001 and r=0.586; p<0.0001 for compact cortex and transitional zone.

CONCLUSION. Pore shape is an independent determinant of bone strength and this beyond total porosity. Strategies to quantify pore shape in clinical and research settings may improve our ability to assess the effects of bone loss and increasing porosity in bone strength in health and disease.

P25
ACCELEROMETER-DETERMINED PHYSICAL ACTIVITY, MUSCLE MASS, AND LEG STRENGTH IN COMMUNITY-DWELLING OLDER ADULTS
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Introduction: The aim of this study was to describe the relationship between accelerometer-determined physical activity (PA), muscle mass and lower-limb strength in community-dwelling older adults.

Methods: 636 community-dwelling older adults (66±7 years) were studied. Muscle mass was measured using dual-energy x-ray absorptiometry whilst lower-limb strength was measured via dynamometry. We measured minutes/day spent in sedentary, light, moderate and vigorous intensity activity using Actigraph GT1M accelerometers.

Results: PA intensity was positively associated with both lean mass percentage and lower-limb strength in a dose-response fashion. Sedentary activity was negatively associated with lean mass percentage but not lower-limb strength. There was a positive association between PA and appendicular lean mass (ALM) in men. There was an interaction between age and activity; as age increased the magnitude of the association of PA with lean mass percentage decreased. Those who adhered to the Australian Department of Health PA guidelines (moderate/vigorous PA >/=150min/week) had greater lean mass percentage, ALM and lower-limb strength.

Conclusion: The amount and intensity of accelerometer-determined PA had an independent, dose-response relationship with lean mass percentage and lower-limb strength. Time spent in sedentary activity was negatively associated with lean mass percentage but was not associated with lower limb strength. The magnitude of the association between PA and lean mass percentage decreased with age suggesting that PA programmes may need to be modified with increasing age.
Aim: The aim of this study was to describe the natural history, predictors of change and structural and clinical factors associated with change in knee cartilage defects over 10 years and three time points.

Methods: 215 participants [mean age 45 (26-61); 58% female] were studied at baseline, 2 and 10 years. Cartilage defects (tibio-femoral and patellar), cartilage volume, bone marrow lesions (BMLs), sub-chondral bone area, meniscal tears/extrusion and effusion were assessed on MRI and joint space narrowing (JSN) and osteophytes on radiographs.

Results: 44% of the participants had at least one cartilage defect at any site at baseline. Most of these remained stable, whereas 26% increased and 13% decreased in severity over 10 years (Fig-1).

Presence ($\beta$=+0.83 (+0.32, +1.60)) and severity ($\beta$ (per grade)=+0.86 (+0.02, +1.70)) of JSN, severity of BMLs ($\beta$ (per unit area)=+0.64 (+0.10, +1.20)) and bone area ($\beta$ (per unit area)= +0.0008 (+0.0002, +0.0014)) independently predicted change in total knee cartilage defects.

Change in cartilage defects was associated with change in BMI, pain, BMLs and cartilage volume loss in the unadjusted analysis but these associations persisted only for BMI ($\beta$= +0.13 (+0.03, +0.25)) and cartilage volume loss (absolute volume ($\beta$= -123.87 (-193.24, -54.50)) and percentage per annum ($\beta$= -0.08 (-0.11, -0.04)) in the fully adjusted model.

Conclusion: Data from this midlife cohort suggests that cartilage defects are on the osteoarthritis causal pathway both for symptoms and structure with sub-chondral bone playing an important role in instigating the cascade of structural abnormalities.
Data on long-term consequences of osteoporotic fracture other than spine and hip are scanty. This study examined mortality risk associated with different types of osteoporotic fracture. Participants in the Canadian Multicentre Osteoporosis Study, a nationwide representative population-based cohort study of Canadians, were followed for 15 years with scheduled interviews and annual postal questionnaires. Incident fractures were identified from annual self-reports and deaths ascertained through contact with a member family or obituary review. 7687 participants (5525 women, 2162 men) aged ≥50 years had one or more subsequent visits. Cox proportional hazards model was used to examine the fracture-mortality association; and Bayesian model averaging method to identify the best prognostic model.

There were 1490 osteoporotic fractures followed by 306 deaths in women (mortality rate: 3.3/100 person-years; 95% CI: 2.9-3.6), and 334 fractures with 96 deaths in men (4.6; 3.8-5.6). The majority of deaths occurred within the first 5 years following hip, vertebral and proximal, but not distal fractures. The risk of mortality was also increased after hip, vertebral and proximal fractures, although not statistically significant for several types of proximal fracture with few participants. The most parsimonious model predicting premature mortality following fracture in both genders included advancing age and current smoking. It also included poor physical health, respiratory diseases and subsequent fracture for women; and poor mental health, diabetes mellitus, stroke, renal disease, and low BMD for men.

Excessive mortality was found following hip, vertebral and proximal osteoporotic fractures. This study highlights the need for early intervention to decrease potentially mortality.

Existing predictive models in osteoporosis have been developed to predict the risk of an initial fracture only. We sought to develop models for predicting the risks of an initial fracture, subsequent fracture, and mortality. This study was based on a population-based cohort consisting of 757 men and 1224 women, aged 60 years and older (average age: 70). Fragility fractures were ascertained using X-ray reports. Mortality was obtained from individual participant during the follow-up period. Femoral neck BMD was measured at the initial visit. A multistate Markov model was used to estimate the transition probabilities between clinical states. Amo...
Trabecular bone score (TBS) is a derived parameter based on grey scale homogeneity which correlates with trabecular microarchitecture and independently predicts fracture risk. TBS recently was incorporated into FRAX®. TBS software is available for Hologic and Lunar densitometers. Previous studies using different scanning modes on Hologic Discovery did not show any significant difference in TBS. GE Lunar acquire spine scans using one of three modes depending on tissue thickness (thin, standard and thick). Thirty patients (mean BMI: 30.8, range 26.2 to 34.1) were identified who had lumbar spine DXA (GE Lunar Prodigy, software 14.10) using standard and thick modes, on the same day. BMD (L1-L4) and TBS were derived from the 30 paired spine scans. There was no significant difference in spine BMD between the two modes. TBS from scans acquired in thick mode were significantly higher than TBS from acquisitions in standard mode (mean TBS difference: 0.24 (20%), SD ± 0.10). Review of acquisition parameters of the modes revealed no difference in pixel size (0.6 x1.05mm), X-ray tube current (3.0 mA) or voltage (76 kV). Spine image acquisition times were however significantly different; 28 seconds in standard versus 56 seconds in thick mode. This would result in lower signal-to-noise ratio in standard mode and consequently reduced image quality. Reduced image quality in standard compared to thick mode would lead to greater inhomogeneity of images and consequently lower TBS.

**Conclusion:** TBS acquired using Lunar Prodigy is dependent on scanning mode used. This could impact on derived FRAX absolute fracture risk.

**P30**

**MULTIPLE DRIVERS OF BONE MARROW LESION DEVELOPMENT IN OSTEOARTHRITIS? MICRODAMAGE, VASCULARITY AND THE METABOLIC SYNDROME**

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1The University of Adelaide, 2Monash University, 3Sa Pathology, 4Royal Adelaide Hospital, 5Repatriation General Hospital

**Aims of the study:** Bone marrow lesions (BMLs) are discrete subchondral bone MRI abnormalities that have potential prognostic value in osteoarthritis (OA). There is limited knowledge of how BMLs develop in OA. Hypothesised causes of BML development include mechanical stress, inflammation or ischaemia. The study aim was to explore the origins of BML development for human knee OA.

**Methods:** Tibial plateaus (TP) and medical histories were obtained from 75 knee OA arthroplasty patients (34-men, 41-women; aged 48-87 years). To identify BMLs in TP, T1 and PDFS-weighted MRI scans were performed. For 56 subjects, TP were micro-CT imaged prior to sampling osteochondral tissue, containing BML/No-BML, for quantitative assessment of microdamage, bone turnover, marrow pathology.

**Results:** MRI detected tibial BMLs for 76% subjects; 24% without BMLs comprised No-BML group. The metabolic syndrome (MetS) was more prevalent for BML (44%) versus No-BML (28%), with higher incidence of type-2 diabetes and hypertension for BML. BML tissue had increased subchondral bone microcrack density (plate and trabeculae, p=0.008), sclerotic microarchitecture (thicker plate, p=0.002; increased trabecular BV, lower SMI, p=0.02), increased osteoid volume/thickness (p=0.005), and increased marrow vascular density (p<0.0001). Marrow inflammation and bone necrosis were not present.

**Conclusion:** Knee OA patients with a tibial BML had an increased prevalence of MetS and its components and the BML tissue showed an altered vasculature, while the increased microcrack burden and adaptive sclerotic bone response of BML tissue supports a mechanical origin for BMLs. These findings suggest multiple possible drivers of BML, which may be markers of OA progression.
**P31**

**BONE MINERAL DENSITY, BONE MASS ACQUISITION AND NUTRITIONAL STATUS IN CHILDREN AND ADOLESCENTS WITH CYSTIC FIBROSIS**

Sharma Sonakshi, Byrnes Catherine, Jaskic Mirjana, Fenwick Sheryl, Cundy Tim

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**Aims:** Improvements in the treatment of cystic fibrosis (CF) have prolonged life expectancy, but other complications have become more prevalent, including low bone density and fractures. We hypothesized that acquisition of bone would be most impaired in the least well children, as judged by their nutritional status.

**Methods:** We measured the BMD at lumbar spine (LS) in 60 CF patients (age 5.9 – 18.8, mean [SD] 12.6 [2.3] years) and calculated the body mass index (BMI) centile. In 38 patients we repeated the BMD 4.0 years after the original scan.

**Results:** At the time of initial scan, mean [SD] BMI z-score was -0.28 [1.38] and mean BMD z-score was -0.92 [1.13]. The two were strongly correlated: r= 0.68, p <0.0001, suggesting that nutritional status was a major determinant of BMD. In the follow up study at a mean age of 16.3 [1.8] years, the height centile was maintained indicating normal growth, but BMI z-score fell significantly (-0.16 to -0.45, p = 0.03). There was a positive correlation between the change in BMI z-score and BMD z-score (r= 0.43, p= 0.007), indicating that patients losing weight were failing to acquire bone normally. We also found a positive correlation between FEV1 z-score and BMD z-score (r= 0.32, p= 0.01), and BMI z-score (r= 0.34, p= 0.008), indicating that patients with poorer lung function were likely to be thinner with lower bone density.

**Conclusion:** Bone acquisition is impaired in the sickest children with cystic fibrosis, as judged by their nutritional status and lung function.

**P32**

**MUSCULOSKELETAL DECLINE AND MORTALITY: PROSPECTIVE DATA FROM THE GEELONG OSTEOPOROSIS STUDY**

Pasco Julie, Mohebbi Mohammadreza, Holloway Kara, Brennan-Olsen Sharon, Kotowicz Mark

Deakin University

**Aim:** To examine the relationship between musculoskeletal deterioration and all-cause mortality in a cohort studied prospectively over a decade.

**Methods:** 750 women aged 50-94yr were followed for a decade after baseline measures of femoral neck bone mineral density (BMD) and appendicular lean mass (ALM) were obtained using DXA, with comorbidities, health behaviours and other clinical measures. The outcome was all-cause mortality from the Australian National Deaths Index. Mortality risks were estimated using Cox proportional hazards models according to BMD-groups (normal-BMD, osteopenia, osteoporosis) and ALM-groups (T-scores >-1 high, -2 to -1 medium, <-2 low).

**Results:** During 6712 person years of follow-up, there were 190 deaths, the proportions increasing with diminishing BMD: 10.7% (23/215) normal-BMD, 23.5% (89/378) osteopenia, 49.7% (78/157) osteoporosis; and with diminishing ALM: 17.0% (59/345) high, 26.2% (79/301) medium, 50.0% (52/104) low. In multivariable models adjusted for smoking, polypharmacy and mobility, compared to those with normal-BMD, mortality risk was greater for those with osteopenia (HR=1.77, 95%CI 1.11-2.81) and osteoporosis (2.61, 1.60-4.24). Similarly, compared to those with high ALM, adjusted mortality risk was greater for medium ALM (1.36, 0.97-1.91) and low ALM (1.65, 1.11-2.45). When BMD and ALM groups were tested together, BMD remained a predictor of mortality (1.74, 1.09-2.78; 2.82, 1.70-4.70; respectively) and low ALM had borderline significance (1.52, 1.00-2.31), further attenuated by smoking, polypharmacy and mobility.

**Conclusions:** Poor musculoskeletal health increased mortality risk. This appears to be driven by a decline in BMD. Low lean mass independently exacerbated mortality risk, and this appeared to operate through poor health exposures.
Scientific Program

Poster Presentations - Clinical

P33

SERUM CARBOXY-TERMINAL TELopeptide of TYPE I COLLAGEN, AS A MARKER FOR SYSTEMICATHEROSCLEROSIS.
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Carboxy-terminal telopeptide of type I collagen (ICTP) is a type I collagen marker produced by matrix metalloproteinase (MMP)-dependent digestion. The fact that atherosclerotic lesions are rich in both type I collagen and macrophages producing MMPs led us to hypothesize that serum ICTP concentrations may be a non-invasive marker of systemic atherosclerosis. Therefore, we examined the association of serum ICTP levels with maximum intima-media thickness (IMT) of carotid arteries, a surrogate index of systemic atherosclerosis, or brachial-ankle pulse wave velocity (baPWV) in patients with atherosclerotic risk factors. We recruited 53 men and 65 women (mean age: 62.8 yr.) without renal failure or bone diseases that are known to affect serum ICTP levels. Patients with more than 1.1mm max IMT, an index of systemic atherosclerosis had significantly higher serum ICTP compared to those with less than 1.1mm max IMT (3.33 ± 0.97 vs 2.82 ± 0.65, p<0.05), and significant positive correlation were observed between serum ICTP and max IMT at p<0.001 and between serum ICTP and baPWV at p<0.05. Furthermore, multivariate analysis revealed that serum ICTP was positively associated with max IMT (p<0.05; 95% CI 0.003 to 0.374). These results suggest that serum ICTP can be used as a non-invasive predictive marker for systemic atherosclerosis.

P34

PREDICTION OF WHOLE BODY LEAN TISSUE MASS FROM SPINE AND HIPS DXA SCANS
Chen Weiwen1, Center Jacqueline2, Freund Judith3, Reiss Tom3, Pocock Nicholas2
1Garvan Institute of Medical Research, 2Garvan Institute of Medical Research, St Vincent’s Hospital Darlinghurst, 3St Vincent’s Clinic Bone Densitometry

Whole body (WB) dual energy X-ray absorptiometry (DXA) is increasingly used to assess body composition. Osteosarcopenia with reduced bone mass and lean tissue (LT) is associated with higher fracture rates than either condition alone. However, the most commonly measured DXA regions remain lumbar spine (LS) and proximal femoral (PF) scans. GE Prodigy software provides an estimation of WB fat % (WBEst Fat %) derived from standard LS and PF scans. We hypothesise that an estimation of WB LT (WBEst LT) can similarly be derived by first estimating WB bone mineral content (WBEst BMC) from regional LS DXA images. Subsequently using WBEst Fat % and weight, WBEst LT could be derived from:

WBEst LT (kg) = Weight (kg) – Weight (kg) x WBEst Fat % - WBEst BMC (kg)

Methods:
131 patients with LS, PF and WB DXA scans obtained on the same day were analysed. To derive WBEst BMC a regression model was developed using L1-L4 BMC, weight and height to predict measured WB BMC from the whole body scans:

WBEst BMC = -2.22+17.4L1-L4BMC+0.01weight+1.69height.

(Goodness of fit: r=0.89, P<0.001)

Results:
WBEst LT was strongly correlated with measured whole body lean tissue. The regression equation is:

Measured WB LT = 0.99 x WBEst LT +1.29  (r=0.97, P <0.0001).

Conclusion: WB LT can be estimated from LS and PF DXA using WBEst Fat %, and WBEst BMC where the latter is derived from LS BMC, height and weight. This provides an opportunity to incorporate LT into fracture risk assessment models without further imaging.
**P35**

**PATELLAR TENDON ENTHESIS ABNORMALITIES AMONGST OLDER ADULTS ARE ASSOCIATED WITH KNEE PAIN, KNEE FUNCTIONAL DEFICIT, AND PATELLAR CARTILAGE VOLUME LOSS BUT NOT SYSTEMIC INFLAMMATION.**

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**Aims:** To determine associations between patellar tendon enthesis (PTE) abnormalities and knee pain, knee functional deficits, systemic inflammation, and cartilage volume loss over 2.6 years in older adults.

**Methods:** Population-based study of randomly selected older Tasmanian adults (n=528; aged 50-80 years). Presence of abnormal bone signal and erosion at the PTE was scored on fat-saturated T2 weighted magnetic resonance images, proximally and distally. Knee pain and function was assessed using the Western Ontario and McMasters University (WOMAC) questionnaire. High sensitivity C-reactive protein (hs-CRP) was performed by immunoturbidimetry assay. Cartilage volume was assessed using manual segmentation at baseline and after 2.6 years. Data was dichotomised at the median and analysed using log binomial or linear regression, adjusted for age, sex, BMI, knee extension, and patellar cartilage defects.

**Results:** Presence of PTE abnormalities was associated with greater knee pain (incidence rate ratio (IRR) 1.21; (95% confidence interval 1.00, 1.48)), poorer knee function (IRR 1.28 (1.04, 1.59)), higher total WOMAC scores (IRR 1.24 (1.01, 1.53)), and greater patellar cartilage loss per annum (β 1.97 (0.42, 3.52)) after adjustment for confounders. Knee bending activity (going up/down stairs) had the strongest association among pain subscales (IRR 2.13 (1.15, 3.94)). PTE abnormalities were not associated with hs-CRP, tibial or femoral cartilage loss.

**Conclusions:** Presence of PTE abnormalities is associated with worse knee pain (predominantly involving the anterior compartment), poorer knee function, worse total WOMAC scores, and greater patellar cartilage loss per annum, suggesting that PTE abnormalities may be important for overall patellar compartment health.

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**P36**

**LOWER LIMB MUSCLE STRENGTH IS ASSOCIATED WITH POOR BALANCE IN MIDDLE-AGED WOMEN: LINEAR AND NONLINEAR ANALYSES**

Wu Feitong, Callisaya Michele, Laslett Laura, Wills Karen, Zhou Yuan, Jones Graeme, Winzenberg Tania

Menzies Institute for Medical Research

Decline in balance begins in middle age, yet the role of muscle strength in balance is rarely examined in this age group. We aimed to determine the association between lower limb muscle strength (LMS) and balance in middle-aged women and investigate whether cut-points of LMS exist that might identify women at higher risk of poorer balance.

Cross-sectional analysis of 345 women aged 36-57 years. Associations between LMS and balance tests (timed up and go (TUG), step test (ST), functional reach test (FRT) and lateral reach test (LRT)) were assessed using linear regression. Potential nonlinear associations were assessed using locally weighted regression smoothing (LOWESS), and cut-points identified using nonlinear least-squares estimation.

Weaker LMS was associated with poorer performance on the TUG [β -0.008 (95% CI: -0.010, -0.005) second/kg], ST [β 0.023 (0.013, 0.034) step/kg], FRT [β 0.071 (0.047, 0.096) cm/kg] and LRT [β 0.028 (0.011, 0.044) cm/kg]. LOWESS suggested nonlinear associations. Cut-points were 29 (95%CI: 24, 33), 47 (28, 66), 50 (14, 85) and 33 (12, 54) kg for TUG, ST, FRT and LRT respectively. The effect size of associations of LMS with balance tests was greater in participants with LMS below the cut-points than those above.

Poor LMS was associated with reduced balance in middle-aged women, particularly in those with LMS below 29-50 kg. These cut-points aid early identification of women at risk of impaired balance due to inadequate LMS, and in whom interventions to improve balance by improving muscle strength might be more effective.
P37
THRESHOLD EFFECTS OF 25-HYDROXYVITAMIN D ON BONE MINERAL DENSITY, PARATHYROID HORMONE AND BONE TURNOVER BIOMARKERS IN CHINESE ADOLESCENTS
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There is no consensus on the definition of vitamin D deficiency for bone health based on serum 25-hydroxyvitamin D (25OHD) levels. This study aimed to determine whether there are thresholds for associations between 25OHD levels and bone outcomes, below which low serum 25OHD have adverse effects on bone health.

This cross-sectional study of 222 healthy Chinese adolescents (aged 12-15 years; 111 girls, 111 boys) measured serum 25OHD, bone mineral density (BMD) of total body, hip and lumbar spine (LS), and serum parathyroid hormone (PTH), bone alkaline phosphatase (BAP) and tartrate-resistant acid phosphatase 5b (TRAP5b).

Prevalence of low 25OHD was 31% (<30nmol/L) and 97% (<50 nmol/L) (mean 25OHD=30 nmol/L). Nonlinear least-squares estimation identified break-points (nmol/L) of 20 (95% confidence interval (CI): 14-27) for total body BMD, 25 (17-34) for hip BMD, 22 (14 -30) for LS BMD and 31 (23-38) for PTH in girls. In boys, break-points (nmol/L) were 39 (24-55) for total body BMD and 35 (27-43) for PTH; no break-points were identified for hip and LS BMD. Below these break-points, greater 25OHD is associated with increased total body BMD and reduced serum PTH, while above them, no such relationship exists. No break-points were identified for BAP in either gender.

Vitamin D insufficiency is common in healthy Chinese adolescents. 25OHD levels of >20-31 nmol/L are required for optimal bone health in girls and 35-39 nmol/L in boys. Influences of calcium intake on thresholds for 25OHD should be addressed in future studies.

P38
QUADRICEPS WEAKNESS AND INCREASED POST-FRACTURE MORTALITY IN MEN AND WOMEN
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Osteoporotic fracture increases the risk of premature mortality. Muscle weakness is associated with fracture and low BMD. However, the role of muscle strength in post-fracture mortality is not well understood. This study aimed to determine the association between muscle strength measured at quadriceps (QS) before and after fracture, and post-fracture mortality.

The study involved 1106 women and 457 men from the Dubbo Osteoporosis Study who had at least one low trauma fracture (ascertained from X-ray reports) after the age of 50. Median follow-up was 12 years (range: 1-26) from time of QS measurement.

During the follow-up period, 687 women and 257 men had QS measured before fracture, and 454 women and 226 men died. In women, each SD (8 kg) decrease in QS was associated with increased mortality of 50% for QS measured before fracture (HR: 1.50; 95%CI: 1.33-1.71) and 64% for QS measured after fracture (HR: 1.64; 95%CI: 1.37-1.95). After adjusting for femoral neck BMD, age at fracture, age at QS measurement, and comorbidities, QS was associated with a 14% (HR: 1.14; 95%CI: 1.00-1.30) and 29% (HR: 1.29; 95%CI: 1.07-1.55) increase in mortality when measured before and after fracture, respectively. In men, after adjustment, each SD decrease (10.5 kg) in QS measured before and after fracture was associated with a 42% (HR: 1.42; 95%CI: 1.17-1.73) and 70% (HR: 1.70; 95%CI: 1.32-2.19) increase in mortality, respectively.

These results indicate that muscle strength, particularly after fracture is a significant predictor of post-fracture mortality. The data suggest that the improvement of muscle strength may benefit post-fracture survival.
P39
VITAMIN D STATUS AND SKIN DAMAGE IN A SUNNY CLIMATE: THE SAFE-D STUDY
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Maintaining adequate vitamin D status without increasing skin cancer risk may be difficult in sunny climates. We therefore examined the relationships between circulating 25-hydroxyvitamin D (25 OHD) levels, ultraviolet (UV) exposure and actinic skin damage in young Victorian women. Safe-D study participants completed an online questionnaire, wore a UV dosimeter for 14 days and attended a site visit. Serum 25 OHD was measured using liquid chromatography-tandem mass spectrometry. Melanin density was measured at the upper, inner arm using spectrophotometry. Actinic skin damage was measured by scoring silicone skin casts of the hand using the Beagley-Gibson grading system on a 1-6 scale (higher score indicating greater damage). Data for 204 participants were available for analysis. Mean (SD) serum 25 OHD was 74.0(28.7) nmol/L. In women with mild skin damage, 23% were vitamin D-deficient (25 OHD < 50 nmol/L) compared to 21% with moderate-to-severe damage (p=0.080). The median skin cast score was 3/6 (Q1-Q3 3-4). Adequate vitamin D status correlated with increasing sun exposure (OR 1.50, p=0.008) and preference to go into the sun to tan when adjusted for season, body mass index (BMI) and melanin density (OR 2.18, p=0.002). Participants with higher melanin density were less likely to have adequate vitamin D when adjusted for season and BMI (OR 0.24, p=0.010). This analysis indicates a high prevalence of moderate-to-severe actinic skin damage in young women despite vitamin D deficiency being common, raising concern whether it is feasible to maintain adequate vitamin D levels while minimising skin damage and skin cancer risk.

P40
BONE TURNOVER MARKERS IN YOUNG WOMEN: THE SAFE-D STUDY
Callegari Emma T1, Gorelik Alexandra2, Garland Suzanne M1, Reavley Nicola1, Chiang Cherie6, Wark John D1
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Interpretation of bone turnover marker (BTMs) relies upon robust normative data but this is limited in young women. The aim of this analysis was to examine the distribution and association of BTMs with relevant covariates. Subjects were 16 – 25 year-old females participating in the Safe-D study. Serum obtained from 312 participants was tested for BCTX and P1NP (Roche Elecsys automated analyser). After excluding 98 participants based on incomplete surveys, medical history, medication use or pathology results, the reference interval (central 95% of normalized values) was 0.2-1.1 ng/mL for BCTX and 16-143 ug/L for P1NP. BTMs were inversely correlated with age and years since menarche (p<0.001 for both). BCTX and P1NP were lower in hormonal contraception users (BCTX: users 0.52 vs. non-users 0.64 ng/mL, P1NP: 61 vs. 78 ug/L, p<0.001 for both). In a gamma regression model, BCTX was correlated with contraceptive use (β = -0.066, p=0.001), lean mass (β = 0.007, p=0.001), fat mass (β = -0.005, p=0.002) and PTH (β = 0.016, p=0.010). P1NP was associated with contraceptive use (β = -0.894, p=0.001), lean mass (β = 0.091, p=0.001) and fat mass (β = -0.056, p=0.007) Restricting the analysis to those aged 20 and above, age and fat mass were no longer associated with P1NP. BTMs were not associated with 25OHD or whole body BMD. To our knowledge, this is the first study to report BTMs in healthy women aged 16 to 25 years; these findings have important application in bone health research and in generating age-specific reference intervals.
P41
GENETIC VARIANTS ASSOCIATED WITH BONE MINERAL DENSITY IN HIP/SPINE ARE NOT RELATED TO OSTEOPHYTES, BONE MARROW LESIONS, BONE MINERAL DENSITY AND AREA IN SUBCHONDRAL BONE
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Aims: To investigate whether bone mineral density (BMD)-associated genetic variants reported in previously published genome-wide association study (GWAS) are associated with subchondral bone-related phenotypes—osteoophytes, bone marrow lesions (BMLs), subchondral BMD (sBMD) and subchondral bone area (sBA), and test the contribution of polygenic effects on these phenotypes.

Methods: 748 participants (mean age, 63 years) from a population-based cohort study who had bone-related phenotypes and genotype data were analysed. Osteophytes (yes/no), BMLs (yes/no), and sBA were measured by X-ray or MRI, and DXA was used to measure sBMD. We selected 58 candidate single-nucleotide polymorphisms (SNPs) with genome-wide significant level previously reported as being associated with hip/spine BMD. Unweighted or weighted genetic risk score (GRS) were constructed from all SNPs related to high BMD in GWAS. Logistic and linear regression was respectively used for binary and continuous variables in univariate and multivariate analysis.

Results: No individual SNPs were associated with osteophytes, BMLs, sBMD or sBA at stringent levels of statistical level (P=0.0009). However, there was a strong association of the GRS with BMD at hip ($\beta=0.574$, $P=3.4\times10^{-8}$) and spine ($\beta=0.757$, $P=7.0\times10^{-9}$) in multivariate analysis with adjustment for age, sex, BMI and principal components of population structure, but not with subchondral bone-related phenotypes.

Conclusion: This study demonstrates that each individual BMD SNP identified in GWAS is not associated with subchondral bone-related phenotypes possibly due to the sample size of this study. Future research in a larger sample is needed.

P42
BROADBAND ULTRASOUND ATTENUATION OF THE CALCANEUS PREDICTS DXA-DERIVED BONE MASS IN CHILDREN
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Broadband ultrasound attenuation (BUA) measured with quantitative ultrasonometry (QUS) is a quick, safe and portable alternate measure of bone health, and is therefore commonly used in paediatric studies. BUA is not, however a direct measure of bone mass, and therefore its validity as a surrogate measure for BMD is sometimes questioned.

Aim: Our aim was to determine the validity of calcaneal BUA as a surrogate for BMD in children.

Methods: Three hundred and eighty-nine boys and girls (age 4-18 years) volunteered to participate. Age of peak height velocity was calculated in order to classify children into pre-, peri-, and post-pubertal groups. BUA was measured at the non-dominant calcaneus (QUS, Lunar Achilles Insight) while BMD and bone mineral content (BMC) were examined at the non-dominant femoral neck (FN), lumbar spine (LS) and whole body (WB) (DXA, XR-800, Norland). Linear regression analyses were undertaken to examine the relationships between BUA and DXA-derived bone mass.

Results: For the whole sample, BUA predicted 29% of the variance in WB BMC and BMD, 23-24% of the variance in LS BMC and BMD, and 21-24% of the variance in FN BMC and BMD ($p\sim 0.001$). BUA predictions were strongest for the most mature participants (pre-pubertal $R^2 = 0.03-0.19$; peri-pubertal $R^2 = 0.05-0.17$; post-pubertal $R^2 = 0.18-0.28$) and marginally stronger for girls ($R^2 = 0.25-0.32$, $p \sim 0.001$) than boys ($R^2 = 0.21-0.27$, $p \sim 0.001$). Conclusions: Our results suggest that calcaneal BUA provides a valid index of bone mass at clinically important sites in children.
P43
INSIGHTS INTO HUMAN CARPAL/TARSAL OSTEOLYSIS SYNDROMES FROM THE STUDY OF NORMAL MURINE CARPAL BONE DEVELOPMENT
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Background: Multicentric carpotarsal osteolysis (MCTO), multicentric osteolysis, nodulosis and arthropathy (MONA) and Winchester syndromes are rare skeletal dysplasias characterised by the progressive loss of the carpal and tarsal bones and the epiphyses of many long bones in childhood. Causative mutations have been identified in MAFB, MMP2 and MMP14 respectively. The skeletal phenotype in MCTO is assumed to be due to pathologic osteoclast-mediated resorption. However, the site-specific distribution of the skeletal phenotype cannot be explained by an indiscriminate increase in osteoclast activity alone. We hypothesized that MAFB, MMP2 and MMP14 have integral roles in carpal/tarsal and epiphyseal bone formation, and that MCTO is a disorder of bone modelling rather than osteolysis.

Methods: Neonatal mouse forepaws were imaged with μCT and both undecalcified and decalcified sections were histologically examined. Immunohistochemistry was used to assess MAFB, MMP2 and MMP14 expression.

Results: μCT showed sequential formation of carpal and distal radius/ulnar ossification centres, comparable to that seen in humans. Carpal ossification, proximal metacarpal ossification and radial/ulnar secondary ossification were morphologically very similar, with a more isotropic arrangement of chondrocytes compared to the columnar organisation of the radial/ulnar epiphyseal growth plates, and a greater degree of cartilage matrix calcification. MAFB, MMP2 and MMP14 were widely expressed in early postnatal chondrocytes and showed similar staining patterns at all time points.

Conclusions: MAFB, MMP2 and MMP14 are widely expressed in neonatal chondrocytes, which suggests these molecules may contribute to carpal-tarsal bone development, providing support for impaired bone modelling as the underlying mechanism of these syndromes.

P44
INCREASED BODY FATNESS HAS NEGATIVE IMPACT ON BONE MINERAL DENSITY IN MIDDLE-AGED AUSTRALIAN WOMEN: THE BUSSELTON HEALTHY AGEING STUDY
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Background: Fat mass index (FMI, fat mass/height²) is a more accurate measure of fatness than BMI. Data regarding FMI-BMD relationships are limited.

Aim: To evaluate associations between FMI, BMI and BMD in participants in the Busselton Healthy Ageing Study.

Methods: Body composition and BMD of hip and total body were measured using DXA in 2,804 participants (1,397 female) aged 45-66 years. Subjects were classified into underweight/fat deficit, normal, overweight/excess fat, obese and severely obese using standard BMI and FMI categories.

Results: BMI and FMI categories were concordant 77.0% of females and 71.1% of males. In females, 9.7% were in a lower FMI than BMI category (low body fat for BMI), whereas 13.3% were in a higher category (high body fat for BMI). For males, the corresponding figures were 15.5% and 13.4%. Compared to those with high body fat for BMI, women with low body fat for BMI were younger (56.7±5.3 vs 55.0±5.3 years, P=0.01), had lower body weight (70.4±8.0 vs 66.7±11.8 kg, P=0.005), not significantly different BMI (26.2±2.5 vs 25.4±4.4 kg/m², P=0.08), and significantly higher femoral neck (0.916±0.132 vs 0.971±0.148 g/cm², P=0.001), total hip (0.952±0.136 vs 1.013±0.152 g/cm², P<0.001) and total body BMD (1.161±0.096 vs 1.200±0.114, g/cm² P<0.001). These associations remained after accounting for age, smoking, alcohol intake and physical activity. Such associations with BMD were not observed in males.

Conclusion: Our study suggests that increased body fatness has a negative impact on bone health in women, and emphasise the importance of maintaining lean mass from middle age.
Poster Presentations - Clinical

P45
CARDIOVASCULAR (CV) RISK FACTORS AND CORONARY ARTERY CALCIUM SCORES (CTCA) IN POSTMENOPAUSAL WOMEN TREATED WITH TERIPARATIDE (TPTH) OR DENOSUMAB (DMAB)

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Aim: Women treated with calcium and anti-osteoporotic agents have been shown to have an increased risk of cardiac events. It remains uncertain whether their risk is due to these therapies per se or to well-known CV risk factors associated with this age group.

Method: We prospectively evaluated 24 postmenopausal women who commenced TPTH after failing anti-resorptive therapies and compared their CV risk to a group of women who were treated de novo DMAB. All women received elemental calcium 600 mg daily if their daily dietary calcium intake < 500 mg and cholecalciferol 1000 IU daily if their serum 25 OHD3 < 50 nmol/L. Investigations were performed at baseline and after 18-month of therapy and included bone density (QCT), bone markers (PINP and u-DPYD/creatinine excretion), CTCA, fasting BSL, serum lipids and plasma homocysteine. Values are expressed as mean ± 1SEM.

Results: The mean age of the group was 73.2 ± 1.9 years (range 55-85). There data are outlined below.

<table>
<thead>
<tr>
<th></th>
<th>TPTH (pre)</th>
<th>TPTH (post)</th>
<th>DMAB (pre)</th>
<th>DMAB (post)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lumbar spine:</td>
<td>mg/mL (T-Score)</td>
<td>68±6.2 (-3.9)</td>
<td>78±10 (-3.4)</td>
<td>80±6.1 (-3.4)</td>
</tr>
<tr>
<td>Femoral Neck:</td>
<td>g/cm (T-Score)</td>
<td>0.55±0.02 (-2.3)</td>
<td>0.58±0.02 (-2.0)</td>
<td>0.65±0.02 (-1.7)</td>
</tr>
<tr>
<td>CTCA: units</td>
<td></td>
<td>177±108</td>
<td>182±112</td>
<td>83±39</td>
</tr>
<tr>
<td>PINP:μg/L</td>
<td></td>
<td>51.9±7.7</td>
<td>120±16</td>
<td>53.1±9.9</td>
</tr>
<tr>
<td>FBSL: mmol/L</td>
<td></td>
<td>5.0±0.1</td>
<td>4.8±0.4</td>
<td>5.4±0.3</td>
</tr>
<tr>
<td>Chol: mmol/L</td>
<td></td>
<td>5.6±0.3</td>
<td>5.5±0.3</td>
<td>5.3±0.4</td>
</tr>
<tr>
<td>Tg: mmol/L</td>
<td></td>
<td>1.6±0.5</td>
<td>1.1±0.3</td>
<td>1.7±0.6</td>
</tr>
<tr>
<td>Homocysteine:</td>
<td>μmol/L</td>
<td>11.9±0.9</td>
<td>13.5±1.3</td>
<td>13±1.9</td>
</tr>
<tr>
<td>Urate: mmol/L</td>
<td></td>
<td>0.34±0.01</td>
<td>0.38±0.02</td>
<td>0.37±0.02</td>
</tr>
</tbody>
</table>

P Value: a <0.01, b <0.001 and c <0.05 versus pre-treatment values.

There were 11 (46%) women on lipid lowering agents, 8 (33%) on anti-hypertensive agents, 12 (50%) with elevated LDL cholesterol levels (>3 mmol/L) and 14 (58%) with elevated homocysteine levels (>10 μmol/L). Seven women (29%) had significantly elevated CTCA scores. A non-significant increase in CTCA was seen in both treatment groups.

Conclusion: Coronary artery disease commonly occurs in this cohort and is associated with increased CV risk factors. While the mean CTCA scores increased in both treatment groups, this was associated with poor vascular risk factor control. Careful CV assessment is needed in this population.
**P46**

**ONSET OF WALKING AND BONE HEALTH: DO EFFECTS PERSIST?**

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**Introduction:** Age at walking predicts bone strength in adolescent males. Here we investigated relationships in late adulthood.

**Methods:** In the Hertfordshire Cohort Study, walking at one year was recorded in 3225 participants. 305 men and 316 women selected on geographic location completed a questionnaire, and underwent bone densitometry assessment, and peripheral QCT examination of the radius and tibia.

**Results:** Mean age was 69.2 (SD 2.5) in men and 69.5 (SD 2.6) in women, with 75% walking by one year. Earlier walking was associated with birthweight (p=0.03 men; p=0.01 women) and talking at one year (p<0.001), and with a lower chance of continuing education (p=0.03), but not with physical activity levels in late adulthood. Male walkers at one year had 0.38 SD (95% CI 0.12, 0.63) lower tibial mass at the 4% site compared with non-walkers, 0.49 SD lower tibial bone density at the 4% site (95% CI 0.24, 0.75) and 0.56 SD lower trabecular tibial density at the 4% site (95% CI 0.30, 0.81) compared with non-walkers with very similar relationships observed at the radius. No such trend was apparent in women. Adjustment for birthweight, age, BMI, social class, current physical activity, calcium intake, smoking and alcohol consumption did not affect these relationships.

**Conclusion:** We found associations between walking by one year of age and epiphyseal bone in the upper and lower limbs of men but not women in their eighth decade of life. Replication studies are required.

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**P47**

**QUANTITATIVE HEEL ULTRASOUND IN BIPOLAR DISORDER: A CASE CONTROL STUDY**

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**Aim:** Bipolar disorder is associated with significant mental and physical disability. Yet, little is known regarding bone health in bipolar disorder. Quantitative heel ultrasound (QUS) is a portable and, importantly, a relatively cost-effective screening instrument to assess bone quality and fracture risk. We aim to investigate bone quality in individuals with bipolar disorder using QUS in a case-control study.

**Method:** Cases were women with a diagnosis of bipolar disorder recruited from the Barwon Statistical Division. Controls, without bipolar disorder, were drawn from the Geelong Osteoporosis Study, a study recruited from the same region. Bipolar disorder was confirmed utilising the Structured Clinical Interview for DSM-IV-TR Research Version, Non-patient edition. Bone quality was measured using the QUS, producing measurements of Broadband Ultrasound Attenuation (BUA), Speed of Sound (SOS) and Stiffness Index (SI). Anthropometry, medication use and lifestyle factors were measured. Multiple linear regression was used to determine differences between the groups.

**Results:** Cases (n=67) were younger and taller (45yrs (39-56) vs 56yrs (43-69), 163.7cm (159.7-169.3) vs. 161.8cm (157.9-166.2), both p<0.05) than controls (n=640). Age and weight-adjusted SI was 5.2% lower (85.3% (95%CI 81.2-89.5) vs. 88.8% (88.5-91.1), p<0.05) in cases compared to controls. Corresponding results for BUA were 106.2dB/MHz (102.5-110.0) vs. 109.8dB/MHz (108.6-111.0), p=0.07, and SOS were 1552.3 m/sec (1544.0-1560.5) vs. 1560.1 m/sec (1557.5-1562.8), p=0.08.

**Conclusion:** These pilot data suggest bone quality is reduced amongst women with bipolar disorder. These findings are similar to findings in other disorders like depression and warrant further investigation as to causes and operative pathways.
P48 INSIGHTS INTO FRACTURES OCCURRING IN PREGNANCY: A REVIEW OF 21 CASES
Herath Madhuni1, Allan Carolyn1, Wong Phillip1, Wallace Euan2, Fuller Peter1, Ebeling Peter1, Milat Frances1
1Department of Endocrinology, Monash Health, 2Department of Obstetrics and Gynecology, Monash Health

Background: There is limited literature examining mechanisms of fracture and outcomes in pregnancy.
Aim: To characterise fractures and associated risk factors at a single tertiary referral centre.
Methods: We audited the Monash Health maternity database (2000-2014) to characterise site, mechanism of fracture, comorbidities and outcomes.
Results: 28 women with fracture in pregnancy were identified (7 excluded with lack of data). The mean age of the remaining 21 women was 28 years (19-42). Minimal trauma fractures (MTF) occurred in 16 (80%) women and fractures secondary to motor vehicle accidents in 5 (8.5%). Of the MTF, fall from standing height resulted in fracture in 14 women (87.5%) and 2 women had rib fractures with coughing (12.5%). Ankle and tibia/fibula fractures occurred in 8 women (50%), while rib and humerus fractures each occurred in 3 women (18.8%). Seven (43.8%) MTF occurred in the second trimester and 8 (50%) in the third trimester. Orthopaedic surgery occurred in 3 women, mostly in the third trimester. One tibia/fibula fracture required repeat surgery and deep venous thrombosis complicated a third trimester fracture. No adverse foetal outcomes were documented. Over one-third (37.6%) of women with MTF had chronic disease: asthma 4/16 (25%), type 1 diabetes 1/16 (6.3%) and hyperthyroidism 1/16 (6.3%). Vitamin D assessments with MTF and medical follow-up occurred infrequently (2 women).
Conclusion: MTF in pregnancy is uncommon but associated with medical comorbidities; 50% involved the lower limb. Coordinated care between medical specialties is recommended for optimal outcome.

P49 MATERNAL HEALTH LITERACY AND CHILD BONE MINERAL DENSITY
Hosking Sarah1, Buchbinder Rachelle2, Brennan-Olsen Sharon1, Hyde Natalie1, Williams Lana1, Pasco Julie1
1Deakin University, 2Monash University

Aim: Maternal health literacy has been shown to play an important role in a variety of child health outcomes but its role in child bone health is currently unknown. We aimed to explore associations between maternal health literacy and child bone mineral density (BMD).
Methods: Data were collected from the 10yr follow-up of the Vitamin D in Pregnancy study. Maternal health literacy was measured using the validated Health Literacy Questionnaire (HLQ). Average HLQ score was split at the mean (3.59 ±0.36; range 2.75-4.44) for analyses. Child areal BMD was measured at lumbar spine and total body less head (TBLH), aged 10-12 (11.04±0.48) years, using dual energy X-ray absorptiometry (Lunar). Age-and-sex-adjusted Z-scores were used for analyses. 155 mother-child pairs had complete measures for maternal health literacy and child BMD.
Results: In child height- and weight-adjusted regression models, maternal health literacy showed a trend for association with spine BMD, but not TBLH BMD (β 0.26±0.13, p=0.05; β 0.08±0.11, p=0.49, respectively). After subsequent adjustment for maternal education, high maternal health literacy was positively associated with BMD at the spine in the children (β 0.28±0.13, p=0.04). Maternal age did not attenuate the associations.
Conclusion: Maternal health literacy was positively associated with child BMD at the lumbar spine. Lower maternal health literacy may be indicative of an increased risk of osteoporosis later in life in children. Whilst further work is warranted, these associations could be related to the maternal role modeling of health behaviours and/or decision making for children during the formative years.
MUSCULOSKELETAL AND HORMONAL HEALTH IN ADULTS WITH CEREBRAL PALSY: NEW OPPORTUNITIES FOR INTERVENTION

Trinh Anne1, Wong Phillip1, Churchyard Andrew2, Brown Justin2, Strauss Boyd3, Fahey Michael2, Ebeling Peter*, Fuller Peter1, Milat Frances1

1Hudson Institute of Medical Research, Monash Health, 2Monash Health, 3Monash University, 4Monash Health, Monash University

Aims: To investigate the relationship between body composition and bone mineral density (BMD) in adults with cerebral palsy and to examine the impact of functional, nutritional and endocrine factors on these parameters.

Method: 45 adults with cerebral palsy attending a single tertiary hospital who had BMD and body composition using dual energy x-ray absorptiometry were identified. Ambulatory status, presence of hypogonadism, use of anticonvulsant medication and PEG feeding was obtained from the medical record. The association between lean tissue mass (LTM), fat, BMD and clinical variables was investigated through univariate and multivariate analyses after adjusting for multi-collinearity.

Results: Mean age was 28.3 ± 11.0 yrs. Ambulatory status was significantly correlated with BMD at all sites (p<0.05). In addition, hypogonadism was predictive for decreased lumbar spine BMD (p=0.006) and PEG feeding predictive for decreased total body BMD. Stepwise linear regression revealed that ambulatory status best correlated with BMD at all sites. Hypogonadism was present in 20% of subjects (see Table). In eugonadal subjects, LTM positively correlated with BMD in both univariate and multivariate analysis; this relationship was not significant in hypogonadal patients where neither LTM, nor fat mass, correlated with BMD.

Conclusion: Low BMD is common in adults with cerebral palsy, especially in those with hypogonadism and reduced ambulation. LTM has a significant positive association with BMD, but its effect is attenuated by the presence of hypogonadism. That early recognition and treatment of hypogonadism in patients with cerebral palsy may have beneficial effects on musculoskeletal health warrants further study.

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<th>Hypogonadal n=9</th>
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<td>Age (yrs)</td>
<td>29.4 ± 11.8</td>
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<td>Male (n)</td>
<td>20 (56%)</td>
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<td>Weight (kg)</td>
<td>58.3 ± 20.2</td>
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<td>Height (cm)</td>
<td>158.2 ± 12.1</td>
<td>143.9 ± 13.6</td>
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<td>BMI (kg/m²)</td>
<td>23.0 ± 6.9</td>
<td>19.4 ± 4.0</td>
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<tr>
<td>Lean mass (g)</td>
<td>35951 ± 10722</td>
<td>25175 ± 7744</td>
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<td>Fat mass (g)</td>
<td>19393 ± 13736</td>
<td>13571 ± 9937</td>
<td>0.370</td>
</tr>
<tr>
<td>BMC (g)</td>
<td>2085 ± 642</td>
<td>1434 ± 498</td>
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<tr>
<td>TBBMD (g/cm²)</td>
<td>1.06 ± 0.11</td>
<td>0.98 ± 0.07</td>
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<tr>
<td>LSBMD (g/cm²)</td>
<td>1.04 ± 0.22</td>
<td>0.77 ± 0.20</td>
<td>0.009</td>
</tr>
<tr>
<td>FNBMD (g/cm²)</td>
<td>0.79 ± 0.17</td>
<td>0.70 ± 0.15</td>
<td>0.314</td>
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<tr>
<td>Anticonvulsant use</td>
<td>18 (50%)</td>
<td>4 (44%)</td>
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<tr>
<td>Non ambulatory</td>
<td>24 (67%)</td>
<td>9 (100%)</td>
<td>0.044</td>
</tr>
<tr>
<td>PEG feeds</td>
<td>3 (9%)</td>
<td>5 (56%)</td>
<td>0.004</td>
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<tr>
<td>Fracture</td>
<td>11 (31%)</td>
<td>6 (67%)</td>
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P51
THE LINK BETWEEN CARDIOVASCULAR RISK FACTORS AND OSTEOPOROSIS
Tabesh Marjan1, Garland Suzanne M.2, Callegari Emma T.1, Gorelik Alexandra3, Rivers Adele1, Nankervis Alison4, Kale Ashwini5, MacLean Skye1, Wark John D.6
1The University of Melbourne, Grattan St, Melbourne, Vic, Australia, 2The University of Melbourne, Grattan St, Melbourne, Vic, Australia and Department of Microbiology Infectious Diseases, Royal Women’s Hospital, Parkville, Melbourne, Vic, Australia, 3Melbourne Epicentre, Royal Melbourne Hospital, University of Melbourne, Parkville, Victoria, 4Department of Diabetes and Endocrinology, Royal Melbourne Hospital, Parkville, Melbourne, Vic, Australia, 5The University of Melbourne, Grattan St, Melbourne, Vic, Australia and Bone and Mineral Medicine, Royal Melbourne Hospital, Parkville, Vic, Australia

Both cardiovascular disease (CVD) and osteoporotic fractures are significant causes of morbidity and mortality. Recent publications identify a correlation between bone mineral density (BMD) and CVD risk factors including metabolic profiles and inflammatory biomarkers. However, it remains unclear how CVD and osteoporosis are linked. The aim of this study was to assess the relationship between CVD risk factors, serum 25-hydroxyvitamin D (25OHD) levels, bone turnover markers (P1NP and BCTX) and BMD in young women.

Safe-D is a cross-sectional study evaluating physical and mental health in 16-25 year-old women recruited through Facebook advertising. Participants completed an extensive online health survey and attended a visit where the above factors and BMD were measured.

Data for 316 participants were analysed. Significant associations between BCTX and HDL ($\beta=-0.353$, p=0.010), lumbar spine BMD and serum insulin ($\beta=-0.183$, p=0.001), femoral neck BMD and total cholesterol ($\beta=-0.173$, p=0.008), 25OHD and HDL ($\beta=0.146$, p=0.008), and femoral neck BMD and triglyceride ($\beta=-0.171$, p=0.007) were observed after adjustment for BMI, smoking, season and age. In addition, 25OHD, BCTX, total hip BMD and femoral neck BMD were significantly associated with body fat percentage ($\beta=-0.111$, $\beta=-0.275$, $\beta=0.200$, $\beta=0.279$, respectively; p<0.001 for all comparisons). No significant associations were found between blood pressure, C-reactive protein, HOMA-IR and other lipids with bone turnover markers, 25OH D and BMD.

These findings suggest that effects of body fat, insulin, HDL, total cholesterol and triglyceride levels may help to explain the association between CVD and osteoporosis. Possible underlying mechanisms warrant further investigation.

P52
DXA TECHNOLOGIST SCAN QUALITY AUDIT
Wormald Jenny, Schultz Christopher
Department of Nuclear Medicine, Pet Bone Densitometry, Royal Adelaide Hospital

The quality of bone density scanning is important in the management of osteoporosis. Poor scanning methods and analysis may result in inappropriate patient treatment. To ensure high standards are achieved and maintained, an audit of DXA technologist scan quality began in 2012 by assessing errors in biographical, scanning, analysis and reporting.

13 technologists performing DXA had 10 scans in 2012 and 2013. In 2014 this was extended to review all scans on one device. Each scan was assessed on the accreditation criteria of the ANZBMS Clinical Densitometry Accreditation process.

The initial results from 2012 demonstrated 4 of 12 staff assessed achieved better than 80% of the expected standard. Two staff had under 50% of the expected standard. Following comprehensive staff re-training, a repeat 10 scan audit in 2013 showed standard rose overall but 3 staff scored less than 50% of the expected.

The relatively low standard resulted in all scans from one DXA scanner being reviewed from 2014 by one of two expert reviewers. This resulted in an average scan/analysis fault rate of less than 10% in 1693 scans. The review process to date in 2015 shows that 17% of 628 studies had errors requiring attention. The majority of these errors were in the lumbar spine and involved bone edges. This achieves better than 80% accuracy rate expected of our staff.

We conclude that an ongoing audit of DXA scanning and analysis is beneficial in achieving the highest standards in clinical practice. Scan audits will be ongoing.
EXERCISE FOR BONE IN CHILDHOOD: DEBUNKING THE PRE-PUBERTY MYTH
Beck Belinda
Griffith University

Background: One of the most commonly espoused notions in the field of exercise and bone is that physical activity will elicit the greatest adaptive response from the skeleton if applied prior to puberty. In actuality, the wide acceptance of the assertion does not reflect the strength of the evidence.

Aim: To identify the source of the misconception and the true balance of evidence for the optimal timing of exercise for bone acquisition in childhood.

Methods: A review was undertaken to 1. identify literature including a claim about the optimal timing of exercise for bone in childhood, 2. scrutinise the detail of research most commonly cited to support the contention that exercise is most osteogenic prior to puberty, and 3. review the balance of the evidence on the optimal timing of exercise for bone in childhood.

Results: The years prior to puberty do not represent a discrete ‘window of opportunity’ to stimulate bone. The primary source of the misperception appears to have been the interchangeable use of the terms ‘pre-puberty’ and ‘pre-menarche’; terms that are clearly not synonymous. There is considerable evidence to suggest that the peri-pubertal years are a more sensitive ‘window of opportunity’ to optimise bone mass in childhood than pre-puberty, but also some evidence to indicate there is no specific ‘window.’

Conclusion: Contrary to conventional thinking, the optimal timing of mechanical loading for bone is unlikely to be prior to puberty. A large-scale exercise trial including children at all stages of maturity would inform the issue.

IMPACT OF PREVIOUS MEDICAL CONDITIONS AND TREATMENTS ON OSTEOPOOROSIS MANAGEMENT: A CASE PRESENTATION
Howard Rowena, Carroll Richard
CCDHB

There are multiple treatments available for the management of osteoporosis that improve BMD and reduce fracture risk. These treatments all have specific associated risks and management is individualised.

Clinical Case: A 46 year old women presented for consideration of treatment for osteoporosis with a history of low impact fracture. Past medical history includes Hodgkins disease as a child treated with surgery and radiotherapy resulting in ongoing infective osteoradionecrosis of her neck with chronic cutaneous fistula. A thyroid nodule 25 years ago showed features suspicious of malignancy on FNA. The patient received ablative radioactive iodine, without surgery and is on suppressive levothyroxine. Menopause occurred at age 44 with ongoing symptoms. Dietary calcium is approximately 1g per day, BMI is 19.6kg/m2 and she partakes in regular weight bearing exercise. DXA shows T-score of -3.2 at left neck of femur and -2.9 at Lumbar spine. FRAX 10 year fracture risk is 5.3% for hip fracture and 8.4% for major osteoporotic fracture.

Management is complicated in this case. Education was given with regards to diet and lifestyle modification. Bisphosphonates and teraparatide are contraindicated due to her osteoradionecrosis and previous radiotherapy. The thyroid cancer risk was deemed low and levothyroxine reduced to replacement dose. HRT was commenced with plan to repeat DXA in 2 years time.

Clinical Lesson: This case illustrates how the treatment of underlying causes of osteoporosis is important, particularly when contraindications to bone specific treatments are present. The relative contribution and management of each of these factors and contraindications will be discussed.
Sample text
The aim of this study is to provide an updated osteoporosis medication persistence analysis which includes the newer non-oral agents, zoledronic acid (IV) and denosumab (SC). Patients from a random 10% sample of the Medicare Australia database were included in this retrospective analysis if they initiated alendronate (weekly), risedronate (monthly/weekly/daily), strontium (daily), raloxifene (daily), zoledronic acid (yearly) or denosumab (6 monthly) between 1 December 2010 and 30 September 2014. Initiation was defined as a 12-month pre-period without any osteoporosis medication. Patients were deemed non-persistent if there were three consecutive months in the follow-up period without a filled prescription. Kaplan-Meier curves were generated based on persistence to the first medication taken and also to “osteoporosis therapy” (i.e. allowing for medication changes). Data from 24,628 patients (76% female, mean age = 73) starting therapy with risedronate (32%), alendronate (28%), denosumab (17%), strontium (12%), zoledronic acid (10%) or raloxifene (1.3%) were analysed. Cessation of all treatments was greatest in the first 12 months followed by a steady decline (Figure 1). Persistence to zoledronic acid at the 12- and 24-month administration time points was similar to that of the oral bisphosphonates and raloxifene. The lowest persistence was observed with strontium. The highest level of persistence was observed with denosumab, which beyond 24 months was at least that of the other agents. This analysis shows that improvement in long-term persistence to osteoporosis therapy in Australia has only been realised with the introduction of denosumab.

**Figure 1:** Kaplan-Meier curves: persistence to initiation medication

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### References

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¹Amgen Australia Pty Ltd, ²Hi Connections Pty Ltd Australia
Scientific Program

Poster Presentations - Clinical

P57
DETERMINATION OF SHORT-TERM PRECISION FOR WHOLE BODY ANALYSIS ON THE HOLOGIC HORIZON A DENSITOMETER.
Nowitz Michael
Pacific Radiology

Aim: To determine the short-term precision for whole body analysis on the HOLOGIC Horizon A densitometer in order to know the least significant change for serial whole body scans.

Methods: In compliance with the Royal Australasian College of Radiologists Standards (July 2014) in vivo short term precision testing was performed after acquiring two new Horizon A densitometers. The machines passed all acceptance tests and were stable on daily phantom quality assessment. With institutional approval and after obtaining informed consent, 30 volunteers had duplicate scans performed by a single operator (PM). An initial scan (then after getting off the scanner and walking about while the scan was analyzed) the second scan. Using the International Society Of Clinical Densitometry’s advanced precision tool, the scan data was recorded and the coefficient of variation (CV) and least significant change (LSC) determined for total fat, lean and bone mineral content (BMC) and bone mineral density (BMD).

Sample statistics:

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<th>Height (Mean/Median)</th>
<th>BMI (Mean/Median)</th>
<th>% Fat (Mean/Median)</th>
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<td>SN 20089</td>
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Results:

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<th>LSC at 95% confidence</th>
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</thead>
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<td>Total Fat</td>
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<tr>
<td>Total Lean</td>
<td>0.52 (281g)</td>
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</tr>
<tr>
<td>Total BMC</td>
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</tr>
<tr>
<td>Total BMD</td>
<td>0.66 (0.007g/cm²)</td>
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<table>
<thead>
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<th>Machine</th>
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<td>Total Fat</td>
<td>0.77 (192g)</td>
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<tr>
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<tr>
<td>Total BMC</td>
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</tr>
<tr>
<td>Total BMD</td>
<td>0.93 (0.010 g/cm³)</td>
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Conclusion:
The Horizon A densitometer provides a high level of precision that will meet exacting clinical applications.
ARE COMPLEMENTARY TREATMENT OPTIONS FOR LYME DISEASE SUITABLE IN ORTHOPAEDIC SURGERY?

Nguyen Van, Tey Nadine
The Royal Melbourne Hospital

Objective: To assess the compatibility of complementary treatment options for Lyme disease with orthopaedic surgery.

Clinical Features: A 40 year old caucasian male was admitted for a right distal tibial fracture, following a motorbike accident. The patient had a history of Lyme disease, diagnosed in 2011 and was treated with complementary therapy, which included intravenous (IV) vitamin C 30g and glutathione 500mg in normal saline, and phosphatidylcholine in 5% glucose infusions. During his admission, the patient had requested continuation of his complementary medicines prior to surgery. The unit pharmacist was referred to review the appropriateness of this therapy and advise the orthopaedic team on a management plan.

Interventions, Case Progress and Outcome: A comprehensive literature review found a correlation between high dose IV vitamin C therapy and increased risk of bleeding and deep vein thrombosis (DVT). The pharmacist advised to withhold all complementary therapy while awaiting surgery. Considering limited evidence on the implications of withholding complementary treatments prior to surgery and the possible risk of bleeding and DVT, it was reasonable to make this recommendation, which was then actioned by all involved in his care. Following a successful open reduction and internal fixation, the patient was discharged home with a general practitioner referral for re-initiation of his complementary therapy.

Conclusions: Patients undergoing surgical procedures are routinely advised on possible complications associated with their surgery. It is important that pharmacists are able to assess the appropriateness and safety of complementary therapies prior to surgical procedures and advise surgeons on medication management plans to optimise patient health outcomes.
FINITE ELEMENT ANALYSIS ACCURATELY REFLECTS THE IMPROVEMENTS IN VERTEBRAL STRENGTH WITH DENOSUMAB IN OVARIECTOMIZED CYNOMOLGUS MONKEYS

Lee David C1, Hoffmann Paul F1, Varela Aurore2, Kostenuik Paul J3, Ominsky Mike S3, Keaveny Tony M4, Peters Karl5
1On Diagnostics, 2Charles River Laboratories Preclinical Services, 3Amgen Inc., 4University of California, Berkeley, CA, USA, 5Amgen Australia

Finite element analysis (FEA) of clinical-resolution computed tomography scans of the hip and spine provides non-invasive measures of bone strength. However, the accuracy of such clinical-type strength estimates for predicting true hip or spine strength has not yet been established after treatment with osteoporosis therapeutic agents. To determine whether FEA can correctly quantify changes in bone strength after treatment with denosumab, vertebra from ovariectomized cynomolgus monkeys (cynos) treated with denosumab (DMab) were subjected to mechanical testing and FEA. After 16 once-monthly injections, T12 vertebral specimens were prepared from three groups: Sham (n=17), OVX+vehicle (n=20), and OVX+DMab (50 mg/kg, n=17). Samples were micro-CT scanned (34 μm voxel size) and tested in compression to failure. Images were coarsened to 300 μm resolution and converted into continuum models having about 30,000 elements, and were then analyzed in a blinded manner using VirtuOst software. Denosumab significantly increased bone strength as determined by both mechanical testing (50±9%) and FEA (46±9%) compared to the Sham group, which was not significantly different than the OVX group (Figure 1). Correlation analysis indicated excellent agreement in normalized strength as measured by mechanical testing and FEA (R²=0.97, p<0.0001, Y=X type of agreement) with no significant differences between the two measurements for any group. These results validate this continuum type of FEA for quantifying treatment effects of denosumab on vertebral strength in this monkey model of postmenopausal osteoporosis. Denosumab-induced improvements in bone strength are driven primarily by the features captured in these models, namely, bone mass and its distribution.
P60
BONE AND BONE MARROW (BM) MACROPHAGES ARE RESISTANT TO LETHAL DOSE IRRADIATION, SELF-REPOPULATE, AND CONTRIBUTE TO HAEMATOPOIETIC RECOVERY IN AN AUTOLOGOUS TRANSPLANT MODEL

Pettit Allison1, Kaur Simranpreet1, Jacobsen Rebecca1, Millard Susan1, Batoon Lena1, Hume David1, Levesque Jean-Pierre1, Raggatt Liza4

1Mater Research Institute - UQ, 2Mater Research Institute and Diamantina Institute - UQ, 3Roslin Institute, University of Edinburgh, 4Mater Research Institute - UQ

We have shown that resident macrophages within bone (osteomacs) and BM play pivotal roles in regulating skeletal homeostasis and haematopoietic stem cell (HSC) niches, respectively. Ontogeny studies indicate that resident tissue macrophages can self-repopulate independent of adult haematopoiesis. Therefore we used an autologous transplantation model to examine whether osteomacs and BM-macrophages are resilient to myeloablation and can self-repopulate. Recipient MacGreen mice (express GFP in myeloid cells) were lethally irradiated and transplanted with congenic B6.SJL enriched HSC. Flow cytometry analyses of BM 2-16 weeks post-transplant showed that monocytes and granulocytes were 100% donor-derived supporting efficient ablation of recipient HSC. In contrast GFP+ recipient BM-macrophages expressing a phenotype consistent with both osteomacs and HSC niche macrophages were detected throughout the time course. A significant 5.9 fold expansion of recipient BM-macrophages occurred between weeks 2 (4.5x10^4 cells/femur) and 5 (27x10^4 cells/femur) post-transplant which coincided with increased BM residence of donor HSC. In situ, GFP+F480+ recipient BM-Macs localized to HSC niche-enriched perivascular microenvironments within both central and endosteal regions. GFP+F480+ recipient osteomacs were also present within periosteum throughout the time course. We selectively depleted recipient BM-macrophages using CD169-diphtheria toxin receptor mice transplanted with enriched congenic GFP+HSC. Depletion of recipient CD169+ macrophages abated donor HSC BM engraftment by 70% and reduced BM reconstitution potential in competitive secondary transplant assays. Overall bone and BM contain myeloablation-resistant self-repopulating macrophages that support haematopoietic recovery post-transplant. These studies also highlight that chimeric models fail to distinguish between resident macrophage and tissue stromal elements in both bone and BM.

P61
OSTEAL MACROPHAGE PHENOTYPE AND DISTRIBUTION IN NATIVE AND INFLAMED PERIOSTEUM

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Characterization of periosteal cell composition is needed to improve knowledge of bone growth and regeneration mechanisms. The importance of osteal macrophages (osteomacs) to bone biology is established but their distribution and phenotype within periosteum has not been specifically investigated. We used immunohistochemistry and flow cytometry approaches to characterise macrophages in native periosteum during murine bone growth and within bone-injury induced activated periosteum and callus. Osteomacs and resident macrophages were distributed throughout resting periosteum. Osteomacs were enriched at sites of periosteal diaphyseal and metaphyseal bone dynamics during normal long bone growth. At simple modelling tracks in diaphyseal periosteum the osteomacs were F4/80+Mac-2-low, which recapitulates endosteal osteomac phenotype at modelling sites. In contrast, many osteomacs within the periosteum at the metaphyseal corticalization zone, a site of complex bone dynamics, had an activated phenotype (F4/80+CD169+Mac-2-ER-HR3+). Activated osteomacs were also present within inflamed native periosteum after bone injury, including adjacent to site of focal endochondral ossification. Mapping of osteomac/macrophage distribution in a pre-clinical femoral fracture model demonstrated enrichment of activated osteomacs/macrophages during the inflammatory and early anabolic phase with transition to homeostatic osteomacs during the late anabolic phase. These observations confirm that osteomacs are key components of both osteal tissues, in spite of salient differences between endosteal and periosteal structure. Demonstration of osteomac activation at sites of complex bone dynamics indicates functional specialization of osteomacs depending on the site-specific bone mechanisms. Overall, osteomac contributions must be consider when investigating the regenerative potential of periosteum.
Glucocorticoid signalling in osteoblasts controls the development of central obesity during ageing

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Central obesity and loss of thermogenic beige adipocytes are hallmarks of ageing but the underlying mechanisms are not well understood. We here demonstrate that glucocorticoid signalling in osteoblasts controls both morphological and functional changes in beige and white adipose tissue in ageing mice.

Glucocorticoid signaling was selectively disrupted in osteoblasts/osteocytes via transgenic (tg) overexpression of 11ßHSD2. Body weight, body composition and adipose tissue function were assessed in female 11ßHSD2-tg mice and litter-matched wild-type (WT) controls at 2, 6, 12 and 18 months of age.

From 2 to 18 months of age, female WT mice gained more in body weight (WT:+28g vs. tg:+13g, p<0.01), overall fat mass (WT:+20.7g vs. tg:+6.3g, p<0.01, Fig.1A) and visceral adipose tissue (WT:+2.8g vs. tg:+0.3g, p<0.01) than their tg littermates. The amount of beige adipose tissue in the visceral fat pads was similar in 2-month-old WT and tg mice. Starting at 6 months of age the metabolic phenotype of the WT and tg mice began to diverge. Remarkably, while WT mice completely lost their thermogenic (beige) adipose tissue as a result of ageing (Fig.1B, left panels), tg animals demonstrated significant amounts of functional beige adipocytes even in old age (Fig.1B, right panels). In keeping with these phenotypical changes, ex-vivo oxygen consumption of visceral adipose tissue decreased with age in WT mice but remained unchanged in tg littermates (WT: -50.4pMoles/min*mg vs. tg: -3.0pMoles/min*mg, p<0.01).

Conclusion: Glucocorticoid signaling in osteoblasts is critically involved in the pathogenesis of age-related obesity and loss of beige adipose tissue.

**Figure 1**

![Graph showing fat mass changes with age](image)
High-fat diets induce obesity and are associated with adverse metabolic outcomes. However, it remains unclear whether these effects are due to the high fat content or the high caloric density of the diet. Here we demonstrate skeletal glucocorticoid signalling contributes to obesity and insulin resistance during high-calorie feeding.

Methods: We used a transgenic (tg) mouse model in which glucocorticoid signalling has been selectively disrupted in osteoblasts/osteocytes via targeted overexpression of the glucocorticoid-inactivating enzyme, 11β-hydroxysteroid dehydrogenase-type 2. Seven-week-old male tg mice and their wild-type (WT) littermates (n=6-15/group) were fed ad libitum a control (13.8kJ/g, 14% total-energy as fat) or a high-calorie, high-fat diet (HC-HF; 16.3kJ/g, 43% total-energy as fat) with 26% total-energy as protein. A group fed a high-calorie standard-fat diet (HC-SF, 16.3kJ/g, 14% total-energy as fat) was included to assess the effects of high-calorie versus high-fat intake. After 18 weeks, body composition, insulin sensitivity and glucose tolerance were measured.

Results: High-calorie feeding, regardless of dietary fat content resulted in significantly increased fat mass in WT mice compared to WT control-fed mice (Fig. A). Furthermore, WT mice fed either high-calorie diet exhibited fasting hyperglycaemia and reduced insulin sensitivity. In addition, mice fed a high-calorie, high-fat diet demonstrated pronounced glucose intolerance (Fig. B, C). Importantly, tg mice with disrupted osteoblastic glucocorticoid signalling were protected from excessive fat accrual (Fig. A) and insulin resistance or glucose intolerance (Fig. D, E).

Our data indicates that high caloric density rather than high dietary fat content is a major driver of metabolic dysfunction. These effects appear to be mediated by glucocorticoid signalling in osteoblasts and osteocytes.
**Poster Presentations – Basic Science**

**P64**
**ROLES OF TRANSCRIPTIONAL FACTOR MOHAWK IN PERIODONTAL LIGAMENT**
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**Background:** Transcriptional factor Mohawk homeobox (Mkx) has been shown to be mainly expressed in tendon tissues and plays an important role in the tissue homeostasis through the regulation of gene expressions such as Col1a1 (Ito et al., PNAS, 2010). Although periodontal ligament (PDL) has compositional similarities with tendons, the function of Mkx in PDL has never been clarified. Thus, the aim of this study is to elucidate the expression and function of Mkx in PDL.

**Materials and methods:** Mkx knockout mice, which were established previously (Ito et al., PNAS, 2010), were analyzed at 10 weeks, 6 months and 12 months of age. The tissue structure around PDL was examined by immunohistochemistry and histological analysis (H-E and toluidine blue stainings). For analysis of collagen fibril formation / microstructure and gene expression of PDL, transmission electron microscopy (TEM) and qPCR were performed.

**Results and conclusion:** We found that Mkx is highly expressed in the PDL of the maxillary molars. In Mkx knockout mice, PDL space of the molar furcation area was expanded more than that of WT, which was accompanied by morphological changes of the cells in PDL. In addition, TEM analysis revealed that collagen fibril of the PDL was abnormally developed in the knockout mice. Finally, we also found that expressions of osteogenic genes were enhanced in PDL of the knockout mice although expressions of PDL-related genes were unchanged. Our results have disclosed that Mkx has an important role in maintenance of PDL.

**P65**
**CILIARY PROTEIN BBS3 IS REQUIRED FOR NORMAL PATTERNING OF CRANIAL BASE.**
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Craniofacial abnormality often reflects the etiology and becomes a key for diagnosing diseases. Bardet-Biedl Syndrome (BBS) is an autosomal recessive disorder caused by dysfunction of primary cilia. BBS patients are reported to manifest craniofacial abnormalities characterized by mid-facial hypoplasia, great width of face and hypodontia. However, the mechanism of generating BBS pathology is yet to be understood. This research aimed to understand the craniofacial morphogenesis of BBS. BBS3 is one of the causative genes of human BBS, which is why we analyzed the craniofacial skeletogenesis of Bbs3/-/- mice by 3D-CT analysis, skeletal preparation and histological analysis.

3D-CT analysis in E18.5 revealed a lack of presphenoid and mid-hypoplasia of basisphenoid in Bbs3/-/- embryos. Their premaxilla were fused in the midline and their longitudinal length of cranial base was shorter compared to wild type. Skeletal preparation demonstrated missing intrasphenoidal synchondrosis with cleaved and laterally expanded cranial base in Bbs3/-/- embryos. Upper incisors were apparently lost in Bbs3/-/- embryos. Histologically, the midline gap in the cranial base of Bbs3/-/- embryos was filled with connective tissue containing fibroblast-like cells. Furthermore, Bbs3/-/- embryos had a fused central upper incisor. The analysis at E14.5 showed that the cleaved cartilaginous template of cranial base was already evident in Bbs3/-/- embryos, and only a single dental lamina was formed in the middle.

Our data suggest that the dysmorphologies observed in the cranial base of Bbs3/-/- embryos may contribute to BBS pathology.
Osteocytes that orchestrate osteoblasts and osteoclasts to adapt bone structure are considered to be the primary victim of the deleterious stress effects such as mechanical loading stress, hypoxia and Glucocorticoids (GCs) administration. We examined the stress response of osteocytes in respond to GCs administration by microarray analyses and revealed REDD1 (regulated in development and DNA damage responses-1) was strikingly induced in primary osteocytes in both mRNA and protein level. Knockdown of REDD1 by siRNA decreased GC induced autophagy in primary osteocytes. Moreover, osteocyte like cell MLO-Y4, over-expressed with GFP-REDD1 without subjecting osteocytes to stress, display a significant up-regulated autophagy suggesting that REDD1 is the key regulator in osteocyte autophagy. We employed IGF-1, one of the activators of mTORC1 by stimulating the kinase Akt to further examine the impact of REDD1 in mTORC1 signaling pathway. Strikingly, in presence of IGF-1, the phosphorylation level of Akt on Thr308 but not on Ser473 is significantly lower, and the phosphorylation of the two Akt major downstream factors, GSK3β and FoxO3a are diminished when REDD1 is over expressed in MLO-Y4 cells. Furthermore, immunoprecipitation of REDD1 showed that REDD1 is interacted with Akt following GC treatment, suggesting that binding of REDD1 to Akt Thr308 is essential for REDD1 to repress mTORC1 signaling. Together, these results suggested that REDD1 is a key regulator in osteocyte autophagy via inhibiting Akt-mTORC1 signalling cascade.

Schmid metaphyseal chondrodysplasia (MCDS) involves dwarfism and growth plate cartilage hypertrophic zone expansion resulting from dominant mutations in Col10a1. Mouse models phenocopying MCDS demonstrated the resultant unfolded protein response (UPR) in chondrocytes involved activation of canonical ER stress sensors, IRE1, ATF6, and PERK with the downstream pathological effect of disrupted chondrocyte differentiation. We investigated the role of the highly conserved IRE1/XBP1 pathway in the pathology of MCDS. Mice with a MCDS collagen X p.N617K knock-in mutation (ColXN617K) were crossed with mice in which Xbp1 was inactivated specifically in cartilage (Xbp1CartΔEx2), generating the compound mutant, C/X. The severity of dwarfism and hypertrophic zone expansion in C/X did not differ significantly from ColXN617K, revealing surprising redundancy for the IRE1/XBP1 UPR pathway in the pathology of MCDS. Transcriptomic analyses of hypertrophic zone cartilage identified differentially expressed gene cohorts in MCDS that are pathologically relevant (XBP1-independent) or pathologically redundant (XBP1-dependent). XBP1-independent gene expression changes included large-scale transcriptional attenuation of genes encoding secreted proteins and disrupted differentiation from proliferative to hypertrophic chondrocytes. Moreover, these changes were consistent with disruption of C/EBP-β, a master regulator of chondrocyte differentiation, by CHOP, a transcription factor downstream of PERK that inhibits C/EBP proteins, and down-regulation of C/EBP-β transcriptional co-factors, GADD45-β and RUNX2. We propose that the pathology of MCDS is underpinned by XBP1 independent UPR-induced dysregulation of C/EBP-β-mediated chondrocyte differentiation suggesting that modulation of C/EBP-β activity may offer therapeutic opportunities.
ANIMAL MODELS OF SKELETAL DISEASE: PRAGMATIC, LOGISTIC AND ETHICAL PROTOCOLS TO INCREASE SKELETAL RESEARCH QUALITY USING 4R PRINCIPLES

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Aims: Yearly over 7 million animals are used for research/teaching purposes in Australia (over 115 million globally), of which a great number are for skeletal research. Commonly, these models are incapable of simulating human conditions [1-3]. We aim at designing pragmatic protocols to update/re-educate skeletal researchers on animal model usage under 4R principles (replacement, reduction, refinement and responsibility).

Materials and methods: To assess the suitability of different animal models for specific purposes and to design protocols for using animals in skeletal research we used: anthropometric, organometric, imaging (HR-pQCT, MicroCT) and histomorphometric data of different animal models and humans (including our pilot research data and those of our collaborators, a comprehensive literature review, veterinary code of practice and field observations.

Results: Pragmatic protocols were designed under the following topics:
R1: REPLACEMENT of lab animals:
1. Mathematical models and pathway analyses (in silico models)
2. Stem cell/tissue/organ culture (in vitro) systems
3. Moribund or already dead animals: how and where to source them
4. Working animals: how and where to source them

R2: REDUCTION of the number of tested animals:
1. Appropriate experimental design: Taking the wrong course from the outset?
2. Collaboration and sample size: Minimising type I and type II errors
3. Publishing all relevant data: The bias of publishing only the results that agree with our hypotheses

R3&4: REFINEMENT of research & promoting the sense of RESPONSIBILITY and altruism:
1. Proper education of the operators
2. Choosing appropriate sensitive and specific methods, rather than “easy” or more affordable ones
3. Using animal models of appropriate size
4. Considering bone and cartilage comparative structure and microstructure in animal models
5. Considering biomechanical data and lifespan of the model animals
6. Considering logistics, and avoiding “easy” models
7. Considering genetic similarity between species
8. Investigating the causes of the disease rather than consequences

Conclusion: Animal usage for skeletal research can be reduced and/or replaced in many cases. Where this cannot be done, research refinement in the hands of a responsible researcher can simultaneously improve the research quality and animal welfare.

References:
Osteoblastic ephrinB2, a receptor tyrosine kinase stimulated by parathyroid hormone, is required for late stage osteoblast differentiation, normal initiation of matrix mineralization and optimal bone strength. As ephrinB2 is also expressed in osteocytes, we tested the effects of osteocyte-specific deletion of ephrinB2 (Dmp1Cre.EfnB2f/f). Three-point bending tests revealed that their bones were brittle compared to controls, but no change in histomorphometric markers of mineralisation were detected. To discover the underlying cause of bone fragility, we used micro-CT and synchrotron-based Fourier-Transform Infra-Red Microscopy (sFTIRM) to assess Dmp1Cre.EfnB2f/f bone matrix composition, in 12 week old females and controls (w/w) (n=13/group).

sFTIRM analysis revealed a 12% higher mineral:matrix ratio in newly mineralised bone at the periosteal edge in Dmp1Cre.EfnB2f/f bones compared to w/w controls. Dmp1Cre.EfnB2f/f bones also had 37% higher carbonate content within the mineral and 13% lower amide I:amide II ratio in maturing bone. This indicates greater collagen stretching along its longitudinal axis, a matrix alteration that may underlie the high mineral and carbonate content, and increased brittleness of Dmp1Cre.EfnB2f/f bones. Indeed, regression analysis with 3-point bending data showed that the ultimate strength of Dmp1Cre.EfnB2f/f bones was determined by the carbonate:mineral ratio; an abnormal relationship that was not upheld in w/w controls.

These data indicates that osteocytes limit collagen stretching during the process of bone mineralisation, to limit carbonate deposition and maintain flexibility of mineralized bone, and that this depends on ephrinB2 expression.

Cdc42 IN NEURAL CREST DERIVED CELLS IS ESSENTIAL FOR PALATAL DEVELOPMENT

Aim & Methods: Craniofacial deformities with multifactorial etiologies such as cleft palate and facial dysmorphism represent some of the most frequent congenital birth defects seen in human. The pathogenesis are often related to cranial neural crest (CNC) wich migrate in a ventrolateral manner as they populate the branchial arches. During CNC cell migration, changes in cell shape and formation, as well as maintenance of subcellular structures as filopodia is dependent on the complex functions of Rho small GTPases. Cdc42, part of the Rho protein family with Rho and Rac, is known to play critical roles in organ ogenesis of various tissues. To investigate the physiological functions of Cdc42 during craniofacial development, we generated cranial neural crest-derived cell-specific inactivated Cdc42 mutant mice (Cdc42fl/fl; P0-Cre).

Results: Most of the Cdc42fl/fl; P0-Cre neonates were viable at birth, though they appeared weaker and no milk was found in their stomachs, and all died within a few days because of suckling and breathing disorders. Cdc42fl/fl; P0-Cre neonates had characteristics of a short face and intracranial bleeding. For anatomical analysis, we demonstrated that these mice had abnormal calcification of the craniums, including frontal, parietal, and interparietal bone, as also seen in micro-computed tomography images. Furthermore, Cdc42fl/fl; P0-Cre neonates demonstrated a cleft palate and there was no fusion of the secondary palate. Histological analyses of Cdc42fl/fl; P0-Cre embryos showed a failure of palatal shelf elongation for the process of palate closure.

Conclusion: Our results suggested that Cdc42 is crucial for facial and palatal organogenesis during craniofacial development.
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INFLAMMATION IS A PREREQUISITE FOR OSTEOPROLIFERATION IN THE PGISp MOUSE MODEL OF ANKYLOSING SPONDYLITIS

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Aim: Ankylosing spondylitis (AS) is a chronic arthritis targeting the axial skeleton. Inflammation is followed by osteoproliferation and ankylosis; however, it is not clear if these processes are sequential or parallel and whether inflammation is required for AS to progress to joint fusion. We investigated disease progression in the proteoglycan-induced spondylitis (PGISp) mouse model of AS which phenocopies many aspects of the human disease.

Methods: PGISp mice were followed over a 40-week time course study. Spinal disease was assessed using a semi-quantitative histological scoring system including inflammation, joint destruction and excessive tissue formation (osteoproliferation). Matrix components were identified using immunohistochemistry. Mineralisation was monitored using computed tomography (CT) images.

Results: Disease initiated with inflammation at the periphery of the intervertebral disc (IVD) at the interface with the longitudinal ligament (enthesis). Inflammation was temporospatially associated with destruction of IVDs, cartilage and bone. Advanced disease was characterised by reduced inflammation, excessive cartilaginous tissue formation which was predominantly proteoglycan enriched matrix with variable type II and X collagen expression. Abnormal mineralisation including bone formation (osteophyte/ectopic bone), joint fusion (cervical vertebral bodies and zygapophysial joints), and mineralisation (IVD and cartilage) was also apparent. Chondrocyte-like cells embedding within a type I collagen positive bone matrix suggested the excessive cartilage was progressing to chondroidal bone.

Conclusion: Disease commences at the periphery of the disc likely at the entheses. Destructive disease progresses through inflammation, disc destruction to pathological cartilage formation with each stage dependent on the previous stage. Finally atypical chondroidal bone formation is involved in the pathological cartilage progresses to bone.

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ADVANCED INTRAVITAL IMAGING OF A CDK1 FRET BIOSENSOR REVEALS TARGETING OF TUMOUR-STROMAL FEEDBACK VIA ROCK INHIBITION IMPROVES GEMCITABINE/ABRAXANE EFFICACY IN PANCREATIC CANCER

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Within tissues, cells are continuously exposed to mechanical stress induced by the surrounding stroma and reciprocate by generating actomyosin-dependent forces by a process called mechano-reciprocity. In pancreatic cancer, an abundant deposition of fibrotic tissue occurs and the tumour-stroma mechanical feedback is enhanced. This contributes to cancer initiation, progression, invasion and metastasis. In particular, the extracellular matrix is stiffer and provides cancer cells with a protective niche.

Here, we hypothesise that targeting Rho kinase-driven ECM stiffness in pancreatic cancer will deprive tumour cells from this protective niche and in turn improve response to chemotherapy. We used 3D organotypic assays – to recapitulate the interactions between cancer cells, stromal cells and the ECM in vitro along with live xenograft and intrasplenic models of pancreatic cancer coupled with state-of-the-art intravital imaging in order to assess how targeting tumour-stromal feedback with the ROCK inhibitor Fasudil improves standard-of-care Gemcitabine/Abraxane treatment. A FLIM-FRET CDK1 biosensor was used in vitro and in vivo to monitor in a live and dynamic manner the spatio-temporal efficacy of chemotherapy following priming of the stroma with Fasudil.

Intravital analysis of CDK1 activity and simultaneous second harmonic generation (SHG) imaging of the ECM demonstrated that targeting ROCK (1) impairs the integrity of the ECM, (2) decreases cancer cell invasion and metastasis and (3) increases the efficacy of Gemcitabine/Abraxane. Our results show that priming of pancreatic tumour with Fasudil disrupts the mechanical tumour-stroma feedback and in turns improves Gemcitabine/Abraxane efficacy. We propose that using FLIM-FRET in this capacity could provide a useful preclinical tool in animal models prior to clinical translation. Furthermore, we suggest that ECM stiffness could be used as a biomarker to identify patients that would benefit from ECM targeting treatment prior to chemotherapy.
Scientific Program

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EVIDENCE THAT OSTEOCYTE PERILACUNAR REMODELLING CONTRIBUTES TO POLYETHYLENE WEAR PARTICLE INDUCED OSTEOLYSIS
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Periprosthetic osteolysis (PO) leading to aseptic loosening, is the most common cause of failure of total hip replacement (THR) in the mid- to long-term. Polyethylene (PE) particulates from the wear of prosthetic liners are bioactive and are implicated in the initiation and/or progression of osteolysis. Evidence exists that cells of the osteoblast/osteocyte lineage are affected by PE particles and contribute to the catabolic response by promoting osteoclastic bone resorption. We hypothesised that osteocytes also contribute directly to PO by removing bone from their perilacunar matrix. Osteocyte responses to ultra-high molecular weight PE (UHMWPE) particles were examined in vitro in human primary osteocyte-like cultures, in vivo in the mouse calvarial osteolysis model, and in the acetabulum of patients undergoing revision total hip replacement (THR) surgery for PO. Osteocytes exposed to UHMWPE particles showed upregulated expression of catabolic markers, MMP13, carbonic anhydrase 2 (CA2), cathepsin K (CTSK) and tartrate resistant acid phosphatase (TRAP). Consistent with this catabolic activity causing perilacunar bone loss, calvarial sections from mice exposed to UHMWPE revealed an increase in osteocyte lacunar area (Lac.Ar) compared to sham-operated animals. Furthermore, acetabular biopsies from patients with PO also showed significantly increased osteocyte lacunar size in trabecular bone adjacent to PE particles, compared with osteocyte lacunar size in bone from primary THR patients. Together, these findings suggest a previously unrecognised action of UHMWPE wear particles on osteocytes, which directly results in a loss of osteocyte perilacunar bone. This action may exacerbate the indirect pro-osteoclastic action of UHMWPE-affected osteocytes, previously shown to contribute to aseptic loosening of orthopaedic implants.

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COMBINED EFFECTS OF DELTA-TOCOTRIENOLS AND LOVASTATIN ON BONE FORMATION
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Osteoporosis is a major public health threat and causing a huge burden. Statins and delta-tocotrienols are potent inhibitors of HMG-COAreductase and have beneficial effects on bone health. However, clinically tolerable doses of statins are required to achieve this biological effect. The current study was designed to evaluate the effect of delta-tocotrienol and lovastatin in combination or individually at clinically tolerable doses on dynamic bone histomorphometry and BMP-2 gene expression in oestrogen–deficient rats. 48 Sprague Dawley female rats were randomly divided into six groups of eight rats: baseline control; sham-operated control; ovariectomised control; ovariectomised+11mg/kg lovastatin; ovariectomised+60mg/kg delta-tocotrienol and ovariectomised+60 mg/kg delta-tocotrienol +11mg/kg lovastatin. Following eight weeks of treatment, the left femur and left tibia were dissected and processed for the analysis of dynamic bone histomorphometry and for the quantification of BMP-2 gene expression. The results showed that long term estrogen deficiency had significantly reduced bone formation. The combined treatment significantly increased double-labelled surface/bone surface (dLS/BS%), mineralizing surface/bone surface (MS/BS%), mineral apposition rate (MAR μm/day) and bone formation rate/bone surface (BFR/BS μm3/μm2/day) and BMP-2 gene expression; and decreased single-labelled surface / bone surface (sLS/BS%). Lovastatin alone was ineffective. The use of delta-tocotrienol alone significantly increased bone formation. However, it was less effective than the combined treatment. Combination of delta-tocotrienol and lovastatin at clinically tolerable doses has the potential to be used as an anti-osteoporotic agent especially in patients who are at risk of both conditions, that is, osteoporosis and hypercholesterolemia. This is especially true for postmenopausal women.
Mutations in p63 gene cause genetic disorders with split-hand/foot malformations in humans, and p63-null mice show severely truncated limbs. Previous studies indicate that these phenotypes are mainly caused by hypoplasia of apical ecto dermal ridge (AER) where p63 is abundantly expressed; however, the underlying molecular mechanisms and specific roles of p63 transcript variants are not fully understood. The present study aimed to reveal specific expressions and roles of each p63 transcript variant in limb development.

FACS analysis of limb bud cells obtained from mouse E11.5 embryos showed that both dNp63 and TAp63 were expressed more abundantly in AER cells than in non-AER cells. In AER-specific p63 knockout mice, forelimb autopods were truncated, and hindlimb zeugopods and autopods were hypoplastic. Expressions of Fgf8 and Jag2, essential molecules in AER, were decreased in limb buds of E11.5 AER-specific p63 knockout embryos. When we introduced dNp63 and TAp63 into mouse ES cells (mESCs), Fgf8 and Jag2 were induced, respectively. Luciferase assay using B16 cells showed that dNp63 enhanced promoter activity of Fgf8, while TAp63 enhanced that of Jag2. Deletion of p63 consensus sequences in these regions significantly decreased both promoter activities, and chromatin immunoprecipitation assay using mESCs confirmed binding of p63 protein to the responsive elements identified in Fgf8 and Jag2 promoters.

Considering that Fgf8 is essential for maintenance of AER and that Jag2/Notch signaling negatively regulates growth and function of AER through apoptosis induction, dNp63 and TAp63 may regulate limb development in different ways through transcriptional induction of different target genes in AER.

Canonical Wnt signaling is important in tooth development but it is unclear whether it can induce cementogenesis and promote the regeneration of periodontal tissues lost due to disease. Therefore, the aim of this study is to investigate the influence of canonical Wnt signaling enhancers on human periodontal ligament cell (hPDLCS) cementogenic differentiation in vitro and cementum repair in a rat periodontal defect model. Canonical Wnt signaling was induced by (i) local injection of lithium chloride; (ii) local injection of sclerostin antibody; and (iii) local injection of a lentiviral construct overexpressing β-catenin. The results showed that the local activation of canonical Wnt signaling resulted in significant new cellular cementum deposition and the formation of well-organized periodontal ligament fibers, which was absent in the control group.

In vitro experiments using hPDLCS showed that the Wnt signaling pathway activators significantly increased mineralization, alkaline phosphatase (ALP) activity, and gene and protein expression of the bone and cementum markers osteocalcin (OCN), osteopontin (OPN), cementum protein 1 (CEMP1), and cementum attachment protein (CAP). Our results show that the activation of the canonical Wnt signaling pathway can induce in vivo cementum regeneration and in vitro cementogenic differentiation of hPDLCs.
Bone is a highly dynamic organ, which is maintained by a balance between bone-resorbing osteoclasts and bone-forming osteoblasts. Increased osteoclast activity shifts the balance toward bone resorption, cause bone destructive diseases such as rheumatoid arthritis and periodontitis. Ectopic induction of receptor activator of nuclear factor kappa-B ligand (RANKL), a regulator of osteoclast differentiation, leads abnormal osteoclastogenesis. For example, in rheumatoid arthritis, synoviocyte is known as a major source of RANKL.

Cholesteatoma is a non-neoplastic lesion arising in middle ear, which consists of hyper keratinizing epithelial layer and fibrous connective tissue. Due to its bone destructive character, it can cause severe complications such as facial nerve palsy or meningitis. However the mechanism of the bone destruction by cholesteatoma remains to be elucidated.

In this study, we established cholesteatoma-like mass composed of mouse ear pinna-derived keratinocytes and fibroblasts on the calvarial bone of mouse. Histological analysis revealed the experimental mass lesion induced osteoclastogenesis on the bone surface. In addition, we succeeded in establishing an in vitro coculture system of keratinocytes, fibroblasts and osteoclast precursors, and found that keratinocytes stimulate the induction of RANKL in fibroblasts, which leads to osteoclastogenesis. Furthermore, we found that IL-17 secreted by keratinocytes elevates RANKL expression in fibroblasts. Thus, our study revealed that interaction between keratinocytes and fibroblasts is involved in regulation of osteoclast differentiation, which may provide the molecular basis for a new therapeutic strategy for cholesteatoma-induced bone destruction.

Osteoporosis is an osteolytic disease which features enhanced osteoclast formation and bone resorption. Identification of agents that can inhibit osteoclast formation and function is important for the treatment of osteoporosis. Dihydroartemisinin is a natural compound used to treat malaria but its role in osteoporosis is not known. We studied the effect of dihydroartemisinin on cell viability by MTS. The effect of dihydroartemisinin on the formation and function of osteoclast was tested by osteoclastogenesis and bone resorption assay. The effect of dihydroartemisinin on the expression of osteoclast maker gene was examined by RT-PCR. The effect of dihydroartemisinin on the RANKL-induced signal pathway was determined by western blot and luciferase reporter gene assays. The OVX mouse experiment was performed to test the effect of dihydroartemisinin on estrogen deficiency-induced bone loss. Here, we found that dihydroartemisinin can suppresses RANKL-induced osteoclastogenesis and bone resorption in a dose dependent manner. Dihydroartemisinin inhibited the expression of osteoclast marker genes such as cathepsin K, calcitonin receptor, and TRAcP. Furthermore, Dihydroartemisinin inhibited RANKL-induced NF-κB and NFAT activity. In addition, using an in vivo ovariectomized mouse model, we show that dihydroartemisinin is able to reverse the bone loss caused by ovariectomy.

Together, this study shows that dihydroartemisinin attenuates bone loss in ovariectomized mice through inhibiting RANKL-induced osteoclast formation and function, indicating that dihydroartemisinin is a potential treatment option against osteolytic bone diseases.
**P79**

**SPARC IS A CRITICAL REGULATOR OF TENDON DEVELOPMENT**

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Secreted protein acidic and rich in cysteine (SPARC) is a matricellular glycoprotein that modulates the interaction of cells with the extracellular matrix (ECM) through its regulation of cell adhesion and matrix assembly. SPARC is widely expressed in bone and tendon. We have found that tendons from adult SPARC−/− mice exhibit smaller collagen fibril reduced tendon mechanical properties and impaired tendon-bone fibrocartilage development. To investigate the role of SPARC in the developing tendon, we examined mice at earlier ages. Tendons of SPARC−/− mice appeared histologically normal compared to wild-type (WT) mice at 1 week of age. In contrast, hypoplastic tendons were seen 3 weeks post-natally, when mice have become actively mobile. The expression of various musculoskeletal developmental transcription factors were also examined in the load-bearing tendon. Consistent with histology, there were no significant differences between 1-week old WT and SPARC−/− mice; however, tendon development transcription factors like Scleraxis and Mohawk were significantly lower in 3-week old SPARC−/− mice. Tendon-derived stem cells (TDSCs) from SPARC−/− mice showed higher cell proliferation, osteogenic and adipogenic differentiation potential but reduced chondrogenic differentiation. We established a tendon development model by applying mechanical stimulation on scaffold-free engineered tendons (SFET) constructed by TDSCs. In this model, WT SFETs develop a tendon-like morphology after 7 days of mechanical stimulation, whereas increased lipid drop formation were observed in SPARC−/− SFETs. These data indicate that SPARC plays a critical role in tendon development by regulating the differentiation of TDSCs.

**P80**

**VESICULIN, AN IGF-II-LIKE PEPTIDE FOR IMPROVING TENDON-BONE HEALING**

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Injuries to tendon-bone interfaces are a significant clinical problem. Healing of these interfaces is particularly problematic due to the complex nature of hard and soft tissue interdigitation. Currently, there are no clinically accepted biological treatments for improving healing of these soft-hard tissue interfaces.

In this study we evaluated vesuculin, a novel peptide structurally similar to IGF-II, for its effects on primary human osteoblasts and tenocytes in vitro. Primary human osteoblasts (HOBs) and tenocytes were isolated from patients undergoing orthopaedic surgery. HOBs and tenocytes were treated with vesuculin, IGF-I or IGF-II. Proliferation of HOBs was assessed by ³H-thymidine incorporation after 24hrs. Treatments were added to tenocyte cultures for 1, 3 and 7 days. Cell viability and expression levels of tendon-related genes were determined using alamarBlue® assays and real-time PCR, respectively.

In HOBs, vesuculin (10⁻⁸M) increased proliferation by ~30% compared to control (p<0.05), similar to both IGF-I and IGF-II. In tenocytes, vesuculin reduced cell viability by ~20% at all time-points (p<0.05). Interestingly, vesuculin at all concentrations (10⁻¹⁰ M, 10⁻⁹ M and 10⁻⁸ M), and IGF-I and IGF-II increased the expression of the tendon-related genes decorin and scleraxis on day 1 (both p<0.05). Collagen Iα1, collagen IIIα1 and tenascin-c expression were unchanged. MMP-3 expression was significantly lowered by vesuculin, but not IGF-I or IGF-II.

Vesuculin is anabolic to osteoblasts, and in tenocytes increases the expression of genes important in tendon biology, particularly scleraxis, a transcription factor necessary for tenocyte development. Overall, vesuculin may hold promise as a novel factor for improving the healing outcomes of tendon-bone repair.
ABSENCE OF VDR IN MATURE OSTEOCLASTS RESULTS IN FOCAL BONE LOSS AND INCREASED OSTEOCLASTIC ACTIVITY WHICH IS DEPENDENT ON AGE AND DIET.

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Mature osteoclasts express the vitamin D receptor (VDR) and cells respond to active vitamin D (1,25(OH)2D3). To assess the role for VDR-mediated activity in osteoclasts in a mouse model, osteoclast-specific vitamin D receptor knockout mice (OclVDR-/-) were generated by mating Cathepsin K-Cre with floxed VDR mice (VDRfl/fl). 6w old male OclVDR-/- mice demonstrated a 12% decrease in vertebral trabecular bone volume (P<0.05) due to decrease trabecular number (Tb.N) and a trend for increased osteoclast number (Oc.N/T.Ar) when compared to VDRfl/fl mice. Despite this, biomarkers such as serum X-laps and TRAP5b were not significantly different between OclVDR-/- and VDRfl/fl mice. Interestingly, RANKL mRNA levels were significantly decreased in OclVDR-/-, suggesting reduced signalling for osteoclastogenesis which occurs by an undetermined feedback mechanism. By 12w of age, no bone phenotype was observed in OclVDR-/- mice under these normal dietary conditions. However, when 3w old OclVDR-/- mice were fed a diet containing restricted levels of calcium (0.03%) and phosphorus (0.08%) for 3 weeks, vertebral bone loss was exacerbated in OclVDR-/- mice resulting in greater resorption per osteoclast which was associated increased cell number and survival. The major finding of the present study is that the absence of VDR enhances osteoclastogenesis and activity can result in increased bone resorption. However, a secondary reduction in RANKL-mediated osteoclastogenesis in vivo may contribute to attenuating bone resorption in OclVDR-/- mice.

ANTI-OSTEOCLASTIC EFFECT OF INTERLEUKIN-3 IS CONSERVED IN RATS

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Interleukin (IL)-3, a pleiotropic cytokine, secreted by activated T cells regulates hematopoiesis. However, its role in bone remodeling is not fully delineated. We have previously shown that IL-3 potently inhibits mouse and human osteoclast differentiation and bone resorption in vitro. To study the role of IL-3 in ovariectomy-induced osteopenia in rats, which is an ideal model of osteoporosis, we first investigated whether anti-osteoclastic action of IL-3 is conserved in rats. We optimized in vitro rat osteoclast differentiation from bone marrow precursors by treating them with recombinant murine receptor activator of NF-κB ligand (RANKL), or recombinant rat tumor necrosis factor (TNF-α). The effect of different concentrations of IL-3 was assessed on the number of tartarate resistant acid phosphatase (TRAP)-positive cells and TRAP activity. We found that IL-3 dose-dependently inhibited both RANKL- and TNF-α- induced osteoclast differentiation in vitro and decreased the TRAP activity. In addition, IL-3 inhibited osteoclast function as assessed by in vitro bone resorption assay. Moreover, IL-3 decreased the expression of osteoclast genes like TRAP, cathepsin K, and integrin β3 as assessed by RT-PCR. IL-3 also inhibits RANKL-induced NF-κB activation by Western blotting. The in vivo anti-resorptive potential of IL-3 on local and pathological bone remodeling in rats by suppression of the ovariectomy-induced ovariectomy respectively is under investigation. In conclusion, the anti-osteoclastic action of IL-3 is conserved across mice, rats, and humans and thus IL-3 could be a potential therapeutic molecule in human bone disorders.
Objective: Assess the prevalence and predictors of low bone density in men and women with more-than-minimal-trauma fractures.

Methods: Men and women attending the outpatient fracture clinic at a tertiary referral hospital in Sydney between March 2006 to October 2014, were offered assessment for osteoporosis. Fractures were classified as minimal trauma defined as a force 'equivalent to or less than a fall from standing height' or more-than-minimal-trauma. Bone mineral density (BMD), the primary outcome of the study, was assessed by dual-energy X-ray absorptiometry at the hip and spine.

Results: Men and women with more-than-minimal-trauma fractures (n=349) had significantly lower BMD than expected for their age, gender and weight (Z-score\(_{\text{spine}}\) = -0.4 SD, 95% CI = -0.5 to -0.3; Z-score\(_{\text{hip}}\) = -0.5 SD, 95% CI = -0.6 to -0.4). Almost 1 in 4 of those over 50 years of age (23%) had osteoporosis. Men and women with more-than-minimal-trauma fractures had similar bone density (Z-score) at both the hip (P=0.25) and the spine (P=0.07) compared to those with minimal trauma fractures (n = 472). The main predictors of low BMD (T-score < -2.0 SD) in those with more-than-minimal-trauma fractures were age over 50 years (OR = 5.72, 95% CI = 3.19 to 10.24), low body weight (BMI < 20 kg/m\(^2\)) (OR= 4.90, 95% CI= 1.73 – 13.90), a prior minimal trauma fracture (OR= 2.66, 95% CI=1.11 – 6.40) and an osteoporosis-associated condition (OR= 2.06, 95% CI= 1.0 – 4.28)

Conclusions: More-than-minimal-trauma fractures, similar to minimal trauma fractures, are associated with lower bone density than expected for age, gender and weight. Men and women over 50 years with a more-than-minimal-trauma fracture should be investigated to exclude low bone density.
A SINGLE PARATHYROID HORMONE (PTH) INJECTION CAN ACCELERATE THE HEALING OF STRESS FRACTURES IN RAT ULNAE.

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Aim: Parathyroid hormone (PTH) has an anabolic effect that can accelerate bone remodelling. Therefore, our aim was to investigate the short-term effect of a single PTH injection on healing of SFx.

Methods: Fifty-two female Wistar rats (300 g) were allocated to PTH and vehicle (VEH) groups. 24 hours after SFx loading. SFx was created in right ulnae of both groups (Figs 1 & 2). Groups were sub-divided into three groups, based on the time ulnae were harvested. Ulnae were harvested two, six and ten weeks after loading. Ulnae were dissected, processed for histology, stained with Toluidine blue and TRAP for osteoclasts count (Figs 3 & 4). Histomorphometry was conducted using Osteomeasure®.

Results: Measurements of cortical area, woven bone area or length of fracture did not differ significantly between the groups. Evidence of trends increasing SFx porosity (resorption), erosion and healing area was observed in PTH groups, with a significant increase in osteoclast number after two weeks (P<0.01). Six weeks post SFx loading, Basic Multicellular Unit (BMU) size, healed bone area, healed bone perimeter, percentage healing, woven bone and healed bone areas were larger in PTH group. Woven bone apposition rate was significantly higher two weeks post loading in PTH group (P<0.01). The increased rate of bone healing continued to rise in PTH group, ten weeks post SFx.

Conclusion: These data suggest that a single PTH injection, 24 hours after SFx initiation, results in active changes in bone remodelling dynamics to accelerate resorption after two weeks and healing after six weeks.

THE ROLE OF JOINT INJURY MECHANICS AND INSTABILITY IN THE DEVELOPMENT OF OSTEOARTHRITIS PATHOLOGY
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The association between anterior cruciate ligament (ACL) injury and osteoarthritis (OA) is attributed to increased joint instability following loss of ACL integrity. This study investigated whether acute changes in biomechanics and/or mechanism of joint injury influence development of OA pathology in preclinical models.

ACL injury was induced in C57Bl6 mice by transection (ACLT) or non-surgical, mechanically-controlled rupture (MCR). Acute change in anterior-posterior joint laxity was measured ex vivo (n=6/injury), and histopathology was compared 2 and 4 weeks post-injury (n=7/injury/time). ACLT and MCR both increased anterior-posterior laxity (p<0.001) but there was no difference between models. Medial femoral and tibial cartilage erosion was worse in MCR than ACLT at both time-points (p<0.01), while in the lateral compartment only tibial cartilage erosion at 4 weeks differed (MCR>ACLT; p<0.05). Cartilage erosion after MCR was always significantly worse in medial versus lateral compartment. Following ACLT there was no medial/lateral difference at 2 weeks, while at 4 weeks femoral cartilage damage was worse laterally and tibial medially (p<0.05). Tibial subchondral bone sclerosis was greater in MCR than ACLT at 4 weeks (p<0.05).

ACL injury results in an acute increase in joint laxity and time-dependent OA pathology. However, the rate and severity of disease was vastly different between injury models despite there being no difference in the initial joint laxity. This suggests that the mechanism of injury and extent of trauma at the time of injury play a greater role in disease development.

CALCITRE SENSING RECEPTOR STIMULATED CANONICAL Wnt SIGNALING IN OSTEOBLASTS IS MEDIATED VIA HOMER-1B/C-mTORC2 COMPLEXES
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Homer scaffolding proteins are known to mediate the formation of signaling complexes with two members of G-Protein Coupled Receptor (GPCR) Family C, mGlur1 and mGlur5, and have well characterized roles in neuromodulation. In human osteoblasts, we show for the first time that the scaffolding protein Homer-1b/c is expressed in these cells. RNAi studies showed that Homer-1b/c was required for CaSR expression, whereas CaSR knockdown enhanced Homer-1b/c expression. Co-immunoprecipitation studies indicate that Homer-1b/c binds to the calcium sensing receptor (CaSR) in a calcium (Ca2+)-dependent manner, and that Homer-1b/c mediates extracellular Ca2+- and CaSR-dependent activation of Akt via the protein kinase mammalian target of Rapamycin complex 2 (mTORC2). RNAi and western blot studies showed that this process results in stimulation of key downstream signaling pathways, such as Akt-dependent canonical Wnt signaling, with increased alkaline phosphatase activity and increased resistance to oxidative stress-induced apoptosis. Homer-1b/c and CaSR are highly expressed in osteosarcoma cells, potentially contributing to a high survival phenotype. Since the CaSR is essential for skeletal development, these new findings reveal Homer-1b/c as a key physiological component of functional and survival pathways in bone-forming cells.
The aim of this ongoing study is to examine, on end-stage knee osteoarthritis (OA) patients, the relationships between knee joint loads measured in vivo using gait analysis prior to knee replacement surgery, and variations in 3D bone microarchitecture of their excised tibial plateau quantified with micro-computed tomography (micro-CT).

Nineteen knee-OA patients (age 67±7 years, mean±SD) underwent pre-operative walking gait analysis: peak external (ERM) and internal rotation moments, knee adduction moment and tibio-femoral joint contact force were determined. After surgery, their tibial plateaus were retrieved and scanned with micro-CT: subchondral bone 3D microarchitecture (bone volume fraction (BV/TV), trabecular thickness, trabecular number and structure model index (SMI)) was analysed in four subregions of interest, in antero-medial (AM), antero-lateral (AL), postero-medial (PM) and postero-lateral (PL) condyles. Subregional microarchitectural differences (repeated-measures ANOVA with post-hoc analysis), and correlations between gait measurements and subchondral bone microarchitecture were examined.

The subchondral bone microarchitecture exhibited statistically significant differences among the four knee subregions (p<0.05): antero-medially, highest BV/TV (up to +86%), trabecular number (+50%), trabecular thickness (+26%), and lowest SMI (-66%) were found, compared to other subregions. The BV/TV in the antero-medial and postero-medial subregions correlated negatively with peak ERM (r=−0.79, p<0.01, and r=−0.59, p<0.01) (Fig.1).

Our results suggest that in knee-OA, during stance, peak ERM is significantly correlated with subchondral BV/TV in the antero-medial and postero-medial tibial plateau, the anatomical locations where BV/TV was highest. This could be linked to microstructural bone adaptation, due to increased stresses in this condyle. Further analysis is ongoing to elucidate these relationships.

Figure 1: (a) *p<0.05, **p<0.01; (b) scatter plot, BV/TV AM vs. peak external rotation moment (ERM); n=19 subjects
**P88**
**INFLUENCE OF ENDOTHELIAL PROGENITOR CELLS ON INTRAORAL WOUND HEALING IN MICE**
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**Objectives:** Intravenous bisphosphonate/chemotherapeutic therapy induces medication-related osteonecrosis of the jaw. Endothelial progenitor cells (EPCs), which are circulating cells that express similar surface markers to vascular endothelial cells, have a therapeutic potential in various diseases. The aim of this study was to investigate whether EPCs affect oral wound healing.

**Materials and Methods:** Saline (VC) and zolendronate (ZA)/cyclophosphamide (CY) were injected to female C57BL/6J mice. A single EPC transplantation (n=7) were carried out just after tooth extraction. Saline (n=7) was used as control for EPC therapy. Maxillae were dissected at 2 weeks after tooth extraction. Histomorphometric, immunohistochemistry and microCT analyses were performed. Independent t-test was used.

**Results:** Impaired wound healing with more necrotic bone and decreased osteoclasts was noted in mice treated with ZA/CY vs. VC treatment only. Wound size was significantly reduced in EPC therapy. Moreover, EPC therapy significantly decreased infiltration of polymorphonuclear cells (PMNs) compared with VC therapy. On the other hand, EPC therapy did not influence hard tissue healing. No alteration of bone mass and necrotic bone was observed by EPC therapy, although osteoclasts were increased via this therapy.

**Conclusion:** EPC therapy accelerates soft tissue healing, but not hard tissue healing in tooth extraction sockets. Promoted soft tissue healing may be associated with decreased PMNs infiltration and upregulated osteoclasts. Hence, EPCs may contribute to alteration of immune responses in soft tissue microenvironment.

**P89**
**A PILOT STUDY EXAMINING THE EFFECT OF 12 WEEKS OF RESISTANCE AND SPRINT CYCLE TRAINING ON BONE MINERAL CONTENT IN VETERAN ENDURANCE CYCLISTS.**
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**Background:** The importance of regular exercise on bone health during aging is widely acknowledged. However, non-weight-bearing activities such as cycling lead to decreases in bone mineral content (BMC) in aging individuals. In contrast, high-impact and strength-training exercises increase BMC in older populations. In aging endurance cyclists concurrent resistance and sprint cycle training may be appropriate to improve or maintain bone health.

**Introduction:** The purpose of this pilot study was to investigate the effects of a 12-week high intensity resistance and sprint cycle training (HIRST) on BMC in veteran endurance cyclists.

**Methods:** Twenty male veteran cyclists (55.0±8.3yr) were divided into two age-matched groups - controls (n=10) who maintained normal endurance training (CTRL) and an intervention group (n=10) who reduced their endurance training volume but added HIRST consisting of twice weekly track sprint training plus twice weekly resistance training at >80% 1RM (EX). Whole body BMC was measured before and after the training block using Hologic ‘Discovery W’ dual-energy X-ray absorptiometry whole body scans which a qualified Clinical Densitometrist analyzed.

**Results:** Paired-sample T-tests revealed EX significantly increased BMC following the 12-week of HIRST (t(9)=3.94, p=0.004) with no change in BMC observed in the CTRL group.

**Discussion:** The results suggest that as little as 12-weeks of HIRST in veteran endurance cyclists may increase whole body BMC.

**Conclusion:** Short term HIRST has shown a positive effect on veteran cyclists BMC possibly improving bone remodeling or formation. To further investigate this preliminary finding, examination of clinically important BMC of lumbar spine and femoral neck regions need to be undertaken.
**P90**

**THE TRANSCRIPTIONAL MODULATOR INTERFERON-RELATED DEVELOPMENTAL REGULATOR 1 IN OSTEOBLASTS MEDIATES BONE REMODELING.**

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The transcriptional modulator interferon-related developmental regulator 1 (Ifrd1) has been identified in various cell types, but little attention has been paid to its role in osteoblast function and bone homeostasis so far. Here, we show that Ifrd1 is a critical mediator of both the cell-autonomous regulation of osteoblastogenesis and osteoblast-dependent regulation of osteoclastogenesis. Osteoblast-specific deletion of murine Ifrd1 increased bone formation and decreased bone resorption, causing high bone mass. Ifrd1 deficiency enhanced osteoblast differentiation and maturation along with increased expression of Runx2 and Osterix (Osx). Coculture experiment revealed that Ifrd1 deficient osteoblasts have higher osteoprotegerin (Opg) expression and less ability to support osteoclastogenesis. Mechanistically, Ifrd1 deficiency increased the acetylation status of p65, a component of NF-κB, via the attenuation of the interaction between p65 and histone deacetylase (HDAC). This led to nuclear export of p65 and a decrease in NF-κB-dependent Smad7 expression. Ifrd1 deficiency attenuated the interaction between β-catenin and HDAC, subsequently increasing the acetylation of β-catenin, leading to its nuclear accumulation and the activation of the β-catenin-dependent transcription of Opg. Collectively, the expression of Ifrd1 in osteoblasts repressed osteoblastogenesis and activated osteoclastogenesis through modulating the NF-κB/Smad/Osx and β-catenin/OPG pathways, respectively. These findings suggest that Ifrd1 has a pivotal role in bone homeostasis through its expression in osteoblasts in vivo and represents a therapeutic target for bone diseases.

**P91**

**IL-4-TREATED BONE MARROW-DERIVED MAST CELLS PRODUCE MICROPARTICLES THAT ACTIVATE REGULATORY B CELLS**

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The ultraviolet (UV) component of sunlight is regarded as a complete carcinogen because it can directly damage DNA and suppress the anti-tumour immune response. UV-suppression of adaptive immunity may also explain the protective effect of sunlight against autoimmune diseases like multiple sclerosis. Mast cells are essential for UV-induced immune suppression, with IL-4 playing a particularly central role in their functional activation. Following exposure to UV, mast cells will migrate into and away from the irradiated skin to the B cell follicles in skin-draining lymph nodes. Because UV is known to activate a subset of B cells with immune suppressive capabilities, we hypothesised that these UV-activated migrating mast cells are involved in the induction of regulatory B cells. To address this, our lab has developed a co-culture system whereby mast cells treated with IL-4 activate phenotypically and functionally suppressive producing microparticles that are capable of inducing regulatory immune modulating cytokines, including IL-10 and IL-13. However, mast cells were the primary producers of these cytokines, not the B cells, suggesting these lymphocytes suppress the immune system through a mast cell-mediated “cross-talk”. Understanding the interaction between mast cells and B cells following UV exposure will allow for the development of potential new therapeutic targets to modulate UV-induced immune suppression.
P92
EFFECT OF ADIPOSE-DERIVED REGENERATIVE CELLS ON IMPAIRED ORAL WOUND HEALING IN MICE
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Objectives: Medication-related osteonecrosis of the jaw is rarely but severely adverse effect in patients taking intravenous bisphosphonate with chemotherapeutic agent. Adipose-derived regenerative cells (ADRCs) have an ability to regenerate various tissues. The aim of this study was to investigate the effects of ADRCs on oral wound healing.

Materials and Methods: Saline (VC) and zolendronate (ZA)/cyclophosphamide (CY) were administered to female C57BL/6J mice. A single ADRC transplantation (n=7) and single injection of recombinant human vascular endothelial growth factor (rhVEGF-C) (n=5) were performed just after tooth extraction. Saline (n=8) was used as control for ADRC and rhVEGF-C therapies. Euthanasia was carried out 2 weeks after tooth extraction. Histomorphometry, immunohistochemistry and microCT scan were performed. Independent t-test was used.

Results: Larger open wound, more necrotic bone and suppressed osteoclasts were noted in ZA/CY treatment compared with VC injection only. ADRC therapy significantly increased bone mass in tooth extraction sockets with increased living bone and decreased necrotic bone, whereas rhVEGF-C therapy did not affect bone. Increased osteoclasts and decreased TRAP positive mononuclear cells (MNCs) were observed by ADRC and rhVEGF-C therapies compared with VC therapy. Enhanced lymphangiogenesis and decreased infiltration of polymorphonuclear cells (PMNs) were also noted in ADRC and rhVEGF-C therapies.

Conclusion: ADRC therapy affected not only bone healing but soft tissue wound healing in tooth extraction socket. Upregulated osteoclast activity by ADRC therapy may promote hard tissue healing. Moreover, upregulated lymphangiogenesis by ADRC therapy may be induced via VEGF-C, resulting in accelerated soft tissue healing.

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DELETION OF G9a, HISTONE METHYLTRANSFERASE, CAUSES IMPAIRED BONE FORMATION
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The expression of cell lineage specific genes during development or cell differentiation is regulated by genetic and epigenetic mechanisms. Epigenetic modifications include the post translational modifications of histone and methylation of DNA. Several types of modifications, such as methylation, acetylation and phosphorylation of N-terminal end of histone are under extensive investigation. Among them, Histone H3 Lysine 9 (H3K9) modifications are proposed to be critical for gene silencing and formation of transcriptional inactive heterochromatin. The methylations of H3K9 are catalyzed by H3K9 methyltransferase, such as G9a. Previously, we have shown the localization of G9a during growth plate chondrogenesis (Ideno et al. 2013), and in the dental mesenchyme during tooth development (Kamiunten et al. 2014). Therefore, we hypothesized G9a plays a role in hard tissue formation. G9a-null mice, however, show embryonic lethality, and the function during osteogenesis and bone formation are unclear. In this study, to elucidate the function of G9a during mesenchymal cell differentiation in vivo, we crossed G9a flox/flox mice with Sox9-Cre mice. We confirmed that Cre expression in neural crest derived and mesenchymal tissue including calvarial bone by lacZ staining of Rosa-lacZ:Sox9-Cre mice. At 3 weeks of age, micro-CT imaging of G9a fl/fl:Sox9-Cre mice showed defective mineralization and significant reduction of skull bone size. Skeletal preparation of G9a-fl/fl:Sox9-Cre mice at E16.5, E18.5 and P1 also showed mineralization defects of calvarial bone. By in situ hybridization and real-time PCR, we observed the reduction of osteocalcin expression in calvarie of G9a-fl/fl:Sox9-Cre mice at E16.5. These results suggest that G9a regulates skull bone formation in vivo.
FEMALES BORN SMALL HAVE BONE DEFICITS DURING LATE GESTATION REGARDLESS OF HIGH FAT DIET

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Intrauterine growth restriction leads to low birth weight and programs adult bone deficits and increased risk of obesity. Obesity leads to inflammation, with increased adiposity known to suppress osteoblast activity. Pregnancy complicated by obesity may further exacerbate these deficits due to inflammation and increased demand for calcium to the developing fetus. We aimed to determine whether a high fat diet (HFD) exacerbates bone deficits during pregnancy in females born small. Uteroplacental insufficiency with consequent pup growth restriction, was induced on embryonic day 18 (E18) in WKY rats using bilateral uterine vessel ligation (Restricted) or sham (Control) surgery (F0 generation). F1 females consumed standard chow or HFD (23% fat) from 5 weeks of age and throughout pregnancy. Females were mated from 20 weeks of age, with femora collected at post-mortem (E20) with pQCT analysis performed.

Pregnant Control and Restricted females consuming HFD had increased dorsal fat mass (+30%) compared to chow-fed counterparts (p<0.05). Restricted females irrespective of diet, had decreased trabecular (-6%) and cortical (-7%) content, cortical area (-7%), periosteal circumference (-5%), bending and torsional strength (-11%) compared to pregnant Control females (p<0.05). Trabecular density was reduced in chow fed females (-7%; p<0.05). Cortical density was not different between groups (p>0.05).

This study highlights that F1 females born small have bone deficits during late pregnancy which may impact on bone health of the subsequent generation; bone deficits were not further exacerbated by HFD. Lifestyle interventions may provide insight to prevent the development of bone deficits during pregnancy.

DISRUPTION TO THE CHONDROCYTE-INTRINSIC CIRCADIAN CLOCK IN HUMAN OSTEOARTHRITIS: AN ESSENTIAL STEP FOR ENABLING THE SWITCH TO THE DISEASED CELL PHENOTYPE?

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Osteoarthritis occurs when chondrocytes undergo a phenotype change leading to excessive cartilage degradation. The molecular events governing this phenotype switch remain unclear. Cell phenotype is partly controlled by the circadian clock. Although the suprachiasmatic nucleus (SCN) clock which synchronises organism behaviour with the day/night cycle is the most well-known, clocks also exist in peripheral tissues. SCN clock dysfunction results in altered cell phenotype and is causatively linked with degenerative disease and cancer. Whether peripheral clock disruption also leads to disease is unknown.

Aim: To determine if the chondrocyte-intrinsic circadian clock is disrupted in osteoarthritis and if clock disruption is causative of the osteoarthritis-associated cell phenotype switch.

Methods: “Healthy” and “osteoarthritic” chondrocytes were isolated from macroscopically normal and visibly damaged cartilage from seven osteoarthritic patients undergoing total knee arthroplasty. Following clock-resetting by serum shock, mRNA levels of the circadian clock components BMAL1, CLOCK, PER1, PER2, CRY1 and CRY2 were measured every 4hrs for 24hrs by real time RT-qPCR.

Results: Peak expression of the circadian transcription factor BMAL1 was eight-fold lower in osteoarthritis compared to healthy chondrocytes whereas peak expression of the BMAL1 antagonist PER2 was two-fold higher (p<0.0001 both). Knockdown of BMAL1 in healthy chondrocytes by RNAi resulted in increased cell proliferation and a five-fold increase in MMP13 expression, features characteristic of the osteoarthritic chondrocyte phenotype.

Conclusions: The chondrocyte-intrinsic circadian clock is disrupted in osteoarthritis. Mimicking this disruption in healthy chondrocytes results in phenotypic changes similar to those seen in osteoarthritis suggesting clock disruption is causative of the disease-associated phenotype change.
**Introduction:** Galanin (GAL) is a naturally occurring neuropeptide which influences cells in multiple ways including migration, apoptosis and differentiation. GAL and its receptors have been found in bone marrow stromal cells (BMSC) and GAL has been shown to increase osteoblast size and number.

The aim of this study was to elucidate the effects of GAL on osteoclasts *in vitro*.

**Methods:** BMSCs treated with 25 ng/mL mCSF and 100ng/mL RANKL were grown for 14 days without GAL (OCL) or with (GAL 10 µM (OCL GAL 10); 100 µM (OCL GAL 100); 1000 µM (OCL GAL 1000)). Analyses performed determined OCL size and number (TRAP stain), GAL receptor localisation (immunocytochemistry) and gene expression (qPCR).

**Results:** All three GAL receptors were localised on OCLs. Treatment of cells with 100 µM GAL significantly decreased size and number of mature OCLs compared to all other groups. This intermediate dose, however, showed a significant increase in expression of RANK, cathepsin K, IL1β and TNF-α.

**Discussion:** This novel study not only localised all three GAL receptors to bone marrow-derived osteoclasts, but has also shown that osteoclastogenesis can be influenced by a carefully considered dose of GAL. Although gene expression of certain osteoclast markers increased, this specific dose of GAL may influence differentiation and fusion of OCL precursors, hence affecting the number of mature, multinucleated osteoclasts. Further research needs to continue to determine resorptive activity and protein translation.

**Conclusion:** GAL limits osteoclastogenesis in a dose-dependent manner, indicating a potential for treatment of skeletal pathologies such as osteoporosis.

**Aim:** Nerve growth factor (NGF) administration enhances fracture healing in animals. NGF acts via its high affinity receptor, trkA. Gambogic amide (GA) is a specific trkA receptor agonist and has greater stability than NGF in relation to systemic administration. Therefore, the aim of this pilot study was to investigate whether systemic administration of GA could facilitate fracture healing in mice.

**Methods:** Bilateral fibular fractures were performed on male C57BL/6 mice. Either 1 mg/kg of GA in DMSO or DMSO (controls) were subcutaneously delivered via mini-osmotic pumps for 2 weeks post-surgery. Gene expression of various osseous markers were measured in 7-, 14-, 21- and 42-day calluses. Callus size and composition were analyzed by micro-computed tomography (µCT) and biomechanical properties of calluses were investigated via three-point bending at 42 days post-fracture.

**Results:** Sclerostin mRNA expression was 2½-fold less in GA-treated calluses compared to controls at 21 days (p = 0.05). µCT analysis showed that both total callus volume and callus mineralized tissue were decreased in the GA-treated group compared to controls at 21 days post-fracture (p < 0.01 and p < 0.05 respectively). By 42 days, biomechanical analyses of calluses showed trends toward an increased Young’s modulus (p = 0.07) and bending stress (p = 0.08) in GA-treated mice compared to controls.

**Conclusion:** GA treatment showed trends towards enhancing fracture healing. It is not clear how GA achieved this, however, the reduction of sclerostin expression in calluses points to one possible mechanism by which this agent may have facilitated healing.
**P98**

**THE BONE PHENOTYPE OF ADIPONECTIN-DEFICIENT MICE**

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Clinical studies found inverse relationships between circulating concentrations of the adipokine adiponectin and bone mineral density (BMD). Laboratory investigations of adiponectin activity in bone produced inconsistent results. The aim of our study was to elucidate the role of adiponectin in skeletal physiology through a comprehensive analysis of the bone phenotype of adiponectin-knockout (APN-KO) mice. Ten wild-type C57BI/6J (WT) and 10 APN-KO (C57BI/6J background) female mice were culled at 8, 14, 21 and 28 weeks and groups of 12 mice at 37 weeks. BMD and body composition were determined longitudinally in the last two groups by DXA. Micro-architecture of femora was analysed by microCT (SkyScan 1172). Bone material properties were determined by nanoindentation and bone strength by three-point bending.

The main differences between the groups were the lower cortical bone fraction in APN-KO at all the time points (P<0.001) and lower cortical thickness from week 14 onwards (P<0.01). Trabecular bone fraction was lower only in young animals (P<0.05). The longitudinal study found lower BMD in APN-KO mice (P=0.04) and a substantial reduction in percentage fat (P<0.0001). Bone material properties and strength were similar in the two groups.

We found that adiponectin deficiency affects bone geometry and BMD negatively, but the differences in bone properties are fairly moderate and do not compromise bone strength. Although adiponectin levels are usually found to be inversely related to fat and bone mass, we found reduced fat and BMD in APN-KO mice. Our study doesn’t support a causative role for adiponectin in mediating fat-bone relationship.

**P99**

**STUDY OF THE EXTRACELLULAR PROTEASE ADAMTS1 AND ITS SUBSTRATES IN THE VASCULATURE: RELEVANCE FOR TUMOUR PROGRESSION AND NEO-VASCULARIZATION**

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Angiogenesis has been under a deep study during last decades. The identification of alternative mechanisms supporting the new vasculature in tumours is being highlighted, with relevance for the appearance of resistance episodes to anti-angiogenic drugs. Among such mechanisms, the classification of tumour cell subpopulations with high plasticity and endothelial-like properties deserves a closer glance. In this scenario, the remodelling of extracellular environment by matrix proteases has turned to be a main modulator, impacting the creation of the neo-vasculature. ADAMTS1, a remarkable member among the extracellular proteases, was firstly identified as an anti-angiogenic molecule. However more recent reports exhibited both pro- and anti-tumorigenic capacities. Importantly, my group revealed the cleavage of extracellular matrix components, Nidogens, molecules with a unexplored role within the perivascular niche. Therefore, my work aims to unveil this functional interaction for tumour plasticity and vascularization regarding healthy and diseased conditions.

I have been able to detect relevant changes in Nidogen deposition in vessels and maturation of the vascular system from a variety of tumour models, subject to ADAMTS1, and relevant for the existence of vascular niches supporting tumour plasticity. Accordingly to the described plasticity of melanoma, I developed several cell lines over-expressing Nidogens. Functional assays confirmed phenotypic alterations regarding capillary-like properties linked with the cleavage of Nidogens. My current studies include the generation of tumours with these cells in mice, already revealing a delay in tumour growth. Supporting this work, the ADAMTS1 KO mouse model expose an important contribution of the protease within the development and tumour progression. At this point I am trying to confirm the importance of the cleavage of these molecules and their deposition in vascular structures. Along with, the evaluation of human melanoma samples and the inclusion of anti-angiogenic therapies within the mouse-tumour model will improve the research with a translational perspective.
TOCOPHEROL AND ASCORBIC ACID PROTECT THE STRUCTURAL AND MATERIAL PROPERTIES OF BONE ALLOGRAFT DURING GAMMA IRRADIATION.

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Despite advances in biomaterials, bone tissues remain important allograft materials in orthopaedics. In total joint replacement (TJR), allografts unite biologically with bone and achieve mechanical properties that provide good long-term clinical outcomes. The quality of bone tissue used for structural allografts is a key factor in long-term success. Gamma irradiation is used to termi- nally sterilise bone allografts. But this approach negatively affects the mechanical properties of bone and subsequently the outcome of revision TJR.

This study sought to determine if the antioxidants, tocopherol and ascorbic acid can preserve the mechanical properties of bone allograft during gamma irradiation.

Ten paired femora were collected from the Queensland Bone and Skin Bank (QBSB), and processed according to QBSB standard protocols. Cortical bone specimens were infused with a mixture of tocopherol and ascorbic acid, and saline (control), for 4 h at room temperature. Control and vitamin-infused bone samples were equally grouped in five gamma irradiation doses: 0, 10, 15, 25, and 50 kGy, and irradiated frozen. Control specimens (0 kGy) remained in a freezer. Cortical bone specimens were mechanically tested by 3-point bending using an Instron 5565A material testing machine.

Cortical bones treated with tocopherol and ascorbic acid provide allografts of equal material and structural quality to non-irradiated bone up to 25 kGy.

Our mechanical data provides evidence to support the use of the combination of antioxidants, tocopherol and ascorbic acid, as a promising radioprotectant to improve the mechanical performance of bone allograft. Improved quality of bone allografts will promote superior clinical outcomes for patients.

A NOVEL OSTEOGENIC ACTIVITY OF VORINOSTAT THROUGH Runx2 STABILIZATION

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Background: Many histone deacetylase (HDAC) inhibitors are well recognized as potential anti-cancer drugs. Inhibition of HDACs induces temporal transcription or epigenetic control, thus regulating many different biological responses. Here, we investigated the osteogenic effect of the HDAC inhibitor suberoylanilide hydroxamic acid (SAHA; vorinostat).

Methods: The effects of SAHA on osteoblast differentiation were examined in the 6XOSE-Luc reporter assay for determination of Runx2 activity and alkaline phosphatase (ALP) activity and in an immunoprecipitation assay to determine the Runx2 acetylation state. The osteogenic activity of SAHA in vivo was studied in an sRANKL-induced osteoporotic mouse model.

Results: SAHA increased the transcriptional activity of Runx2 in a dose-dependent manner in the 6XOSE-Luc reporter assay. SAHA by itself was unable to induce ALP activity; however, SAHA enhanced ALP activity induced by BMP-2. The degree of acetylation of Runx2 was increased with SAHA treatment, which suggests that the increase in Runx2 transcriptional activity might be dependent on stabilization by acetylation. Also, SAHA successfully reversed sRANKL-induced osteoporotic bone loss.

Conclusions: Our study shows an intriguing osteogenic potential of SAHA in a BMP-2-dependent manner and suggests that SAHA could be used at lower doses along with BMP-2 to treat osteoporosis.
Glucocorticoid (GC) excess has significant adverse effects on fuel metabolism which often limits their therapeutic use. Our current research shows that male mice are more susceptible than females to GC-induced metabolic dysfunction. We aimed to determine whether this difference in metabolic response was due to an interaction of GCs and testosterone.

Eight-week-old intact male and castrated male mice received placebo or corticosterone (CS) at a dose of 50µg/ml in the drinking water for 4 weeks. Body composition, fat-pad weights and insulin tolerance were assessed.

Intact-male mice rapidly developed insulin resistance within just one week of CS treatment, whereas castrated-males remained completely insulin sensitive even after four weeks of CS. Similarly, the profound obesity seen in intact-males in response to CS treatment was absent in castrated-males (Fig.A). Following 4 weeks of CS treatment, retroperitoneal, inguinal, interscapular and gonadal fat pad mass had all increased in size in CS-treated intact males but not in castrated mice (Figs.B-D). To test whether testosterone was sensitizing males to the adverse metabolic effects of CS, the same experiment was performed in castrated males receiving dehydrotestosterone (DHT). Interestingly, in the presence of DHT treatment, CS again induced severe insulin resistance and obesity. Although DHT alone induced a 38% increase in overall fat mass (Fig.A), this effect was site specific with only the gonadal fat pad increasing in size. In contrast, CS treatment in DHT-replaced mice increased all fat depots to the same extent as in CS treated intact-males.

This data indicates that testosterone mediates the development of glucocorticoid-induced metabolic dysfunction.
P103
GENDER DIFFERENCES IN THE METABOLIC RESPONSE TO GLUCOCORTICOIDS - THE ROLE OF THE OSTEOBLAST

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The prevalence of autoimmune conditions is higher in women and often requires high dose glucocorticoid treatment. Glucocorticoid (GC) excess has significant adverse effects on fuel metabolism which are partially mediated via the osteoblast. We made the surprising finding that female mice are more resilient than males to the adverse metabolic effects of GCs. We used a transgenic (tg) mouse model in which GC-signaling has been selectively disrupted in osteoblasts/osteocytes via overexpression of the GC-inactivating enzyme, 11βHSD2. Eight-week-old male and female wild-type (WT) and tg mice received placebo or corticosterone (CS) at a dose of 75µg/ml in the drinking water for 4 weeks. Body composition, insulin tolerance and osteocalcin concentrations were assessed.

While CS treatment caused profound insulin resistance in WT-male mice, tg-males were partially protected from the development of insulin resistance. In contrast, WT-females developed only mild insulin resistance while tg-females remained completely insulin sensitive. Similarly, CS induced a 70% increase in fat mass in WT-males, which was attenuated in tg-males at 23% (p≤0.001). Fat gain was significantly less pronounced in WT-females than WT-males (26% vs. 70%; p≤0.001) while tg-females did not accrue fat following CS treatment. In line with the observed phenotypic and metabolic differences, osteocalcin serum concentrations were higher in CS-treated female WT and tg mice than in their respective male counterparts.

Disruption of GC-signaling in osteoblasts attenuated the adverse metabolic effects of CS in both genders. However, female mice were more resilient to GC-induced dysmetabolism than male animals, possibly due to maintenance of higher circulating osteocalcin levels.

P104
CDH1/CDH2 PEPTIDE/PROTEIN-FUNCTIONALIZED POLYURETHANE SUBSTRATES INDUCED OSTEOGENIC DIFFERENTIATION FOR HUMAN MESENCHYMAL STEM CELLS

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Cell-cell interactions mediated by cell adhesion molecules play an important role in controlling stem cell differentiation into multiple tissue endpoints. In terms of bone formation, the cell adhesion molecules N- and E-cadherin are prime candidates for directing bone marrow-derived mesenchymal stem cell fate, given their changing expression in osteoblasts during development. Degradable polyurethanes (dPUs) have been widely used for biomedical applications but intrinsically have limited potential for bone regeneration due to them lacking osteo-conductive or inductive cues. In this research, bio-dPU has been synthesised using a simple two step procedure. Firstly, we generated a prepolymer using polycaprolactone diol as soft segment, and hexamethylene diisocyanate as hard segment, and then followed with the addition of butanodiol, as chain extender. The terminal hydroxyl group on the polyurethane backbone was thereafter converted into tosylate by a nucleophilic substitution, which is an intermediary group for the generation of azide-functionalised dPU polymers. After forming films from the azide functionalised dPU, the azide groups were reacted with an alkyne-Ecadherin peptide (dPU-CDH1) by copper catalyzed Huisgen 1, 3-dipolar cycloaddition. Additionally, recombinant human N-Cadherin (CDH2) was physisorbed onto the dPU surface (dPU-CDH2) in order to compare each CAM variant and their impact on osteogenic differentiation. In vitro cell culture experiments utilizing human Mesenchymal Stem Cells (hMSCs) on these surfaces showed enhanced cell adhesion, proliferation and osteogenic differentiation in a time-dependent manner. At both day 14 and 21, Alizarin Red S staining showed that both dPU-CDH1 and dPU-CDH2 polymers significantly enhanced mineralization nodule formation for hMSCs when compared to controls. RT-PCR results demonstrated that both dPU-CDH2 and dPU-CDH1 polymers significantly enhanced bone-related gene expression (RUNX2, OCN, OPN and ALP) and bone matrix proteins (ALP and OPN) of hMSCs at day 7, 14 and 21 when compared to that of dPU-alone polymers. Furthermore, Wnt-related genes (WNT3a, LRP5, AXIN2 and CTNNB) were increased on dPU-CDH surfaces when compared to dPU-alone polymer. Our data suggest that cadherin-mediated surface modification of dPU-polymer potentiates the regenerative capacity of human stem cells for bone tissue engineering.
P105
HISTOLOGICAL EXAMINATION ON BONE MATRIX SURROUNDING OSTEOCYTIC LACUNAE IN MICE WITH PTH ADMINISTRATION AND IN LACTATING MICE FED WITH CALCIUM INSUFFICIENT DIET
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Introduction: In this study, we have attempted to histologically verify “osteocytic osteolysis” proposed by Bélanger in 1960’s.

Materials and Methods: Wild-type (Wt) ICR and Rankl-/− male mice were injected with hPTH (80µg; 1-34), and then, bone matrix surrounding the osteocytes was examined under TEM, nanoindentation of AFM, confocal laser microscopy and isotope microscopy using 42Ca or 44Ca. In addition, we investigated osteocytic lacunae in lactating mice fed with Ca insufficient diet with or without administration of alendronate for 10 days.

Results and Discussion: At six hours after PTH administration, enlarged osteocytic lacunae were observed in the Wt and Rankl−/− cortical bone, and von Kossa staining demonstrated demineralized bone matrix surrounding the osteocytes. Under TEM observation, there are some fragmented collagen fibrils and pieces of mineralized matrices in enlarged and irregularly-shaped osteocytic lacunae. Calcein labeling and 42Ca isotope was detected on the walls of some osteocytic lacunae. Lactating mice fed with Ca insufficient diet with alendronate administration, consistently, showed the enlarged osteocytic lacunae, and sometimes demonstrated labeling of calcein and 42Ca. It seems likely, therefore, that osteocytes enable to erode the surrounding bone matrix and deposit minerals on their lacunae.

P106
REGULATING OSTEOCLASTIC BONE RESORPTION BY HISTONE DEACETYLASE 1 AND 2 INHIBITION
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Osteoclasts are necessary for bone removal during the remodelling process. However, unregulated osteoclast activity can lead to bone loss pathologies. Targeting enzymes involved in modifying gene expression or protein function, histone deacetylases (HDACs), is an investigative and clinical tool for regulating cell differentiation and activity. Specific HDACs are elevated during osteoclast formation. Selectively targeting these may be useful for regulating osteoclast activity. Previous studies identified anti-bone resorptive effects of broad acting HDAC inhibitors (HDACi). However, the roles of specific HDACs are currently unknown. The aim of this study was to target individual HDACs during osteoclastogenesis to identify the key HDACs regulating osteoclast development and activity.

Human osteoclast progenitors were isolated from donor buffy coat (n=12) obtained from the Australian Red Cross. Osteoclast were differentiated by RANKL addition (10nM), and their formation confirmed by TRAP staining and pit resorption of dentine. HDACi targeting HDACs 1, 2, or 5 were used (100nM) alone or in combination to identify their suppressive capabilities on osteoclast formation, activity and expression of osteoclast related genes.

Individual HDACi had no significant effect on the formation or activity of cultured osteoclasts. However, combining HDAC 1 and 2 inhibition suppressed resorption (p=0.018). Variations in the expression of osteoclast genes were also seen amongst treatment groups. Further analysis is required to determine a mechanism of HDAC 1 and 2 regulation of osteoclast activity.

This study identified possible redundancy in HDAC control over resorption, which can be exploited using inhibitors targeting multiple HDACs for the management of bone loss
Melphalan is a cytotoxic chemotherapeutic used to treat multiple myeloma, a plasma cell cancer, which causes osteolytic bone disease. However, the effect of melphalan on bone metabolism is currently unknown. We previously reported that some chemotherapeutic agents increase osteoclast formation in a cell stress-dependent manner. We thus investigated whether melphalan promotes osteoclast formation and causes bone loss.

Murine bone marrow cells and RAW264.7 cells were treated with RANKL and melphalan (0.2 to 2µM) in the presence or absence of the cell stress response inhibitor KNK437 (10µM) and effects on TRAP-positive osteoclast formation was determined. The effect of melphalan on markers of osteoclast differentiation and stress was determined by qRT-PCR, immunoblot, and/or reporter assays. 8-week old male mice (C57Black6/Kalwrij) were treated with melphalan (2mg/kg) or vehicle 3 times/week via intra-peritoneal injection for 2 weeks and effects on bone structure were determined by microCT analysis.

Melphalan treatment dose-dependently increased osteoclast formation in bone marrow cells and RAW264.7 cells (5.3 fold at 1µM and 3.7 fold 2µM, p<0.001, respectively); KNK437 treatment abolished these effects. Melphalan increased expression of TRAP, DC-STAMP, OC-STAMP, and cell stress marker, HSP70. However RANKL-induced NFκB or NFATc1 signals were unaffected although melphalan increased MITF levels. Mice treated with melphalan showed decreased trabecular bone volume and trabecular number (by 50.9% and 46.3%, respectively, both p<0.01); trabecular thickness was unaffected.

Taken together these data suggest that melphalan increases osteoclast formation and bone loss in a cell stress dependent manner, which may exacerbate bone destruction seen in patients with multiple myeloma.
P109
CALCIUM-SENSING RECEPTOR (CaSR) CONTROL OF CYP27B1 EXPRESSION: ROLE OF PKC AND MEK
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Extracellular Ca²⁺ (Ca²⁺₀) has opposing effects on CYP27B1 gene expression in different tissues, and thus on local 1,25-dihydroxyvitamin D synthesis. However, the underlying mechanisms remain unknown. The present study investigated the role of the CaSR in Ca²⁺₀-mediated regulation of CYP27B1 expression using CYP27B1 promoter-luciferase constructs transfected into control HEK-293 cells, or HEK-293 cells that express the CaSR (HEK-CaSR cells). We previously showed a Ca²⁺₀-dependent biphasic response that peaked at around 3.0 mM Ca²⁺₀, which was absent in control HEK-293 cells. These responses were left shifted by cinacalcet (1.0 μM), and inhibited by NPS 2143 (1.0 μM), but this inhibition appears to be overcome at 5.0 mM Ca²⁺₀. The data identifies the 305 bp proximal region of the CYP27B1 promoter to be sufficient for activation by 3.0 mM Ca²⁺₀. However, there is a loss of Ca²⁺₀-dependent inhibition. Preliminary data also suggest the involvement of MEK and PKC isoforms in regulating CYP27B1 expression. The major finding of the present study is that the CaSR mediates Ca²⁺₀-dependent activation, and inhibition of CYP27B1 expression via multiple signalling pathways in HEK-CaSR cells. Further studies in cells that endogenously express the CaSR and CYP27B1 may be used to elucidate the pathways involved in activation, and/or inhibition under a physiological context.

P110
SKELETAL DYSPLASIA CAUSED BY THE TRPV4 V620A MUTATION
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Although mutations of Ca²⁺ₐ-permeable nonselective cation channel, Transient Receptor Potential channel Vanilloid 4 (TRPV4), are known to be associated with human skeletal disorders, including brachyolmia, platyspondyly and kyphoscoliosis, similar skeletal defects in mouse models of the mutation have not yet been characterized.

To determine whether TRPV4 mutations cause skeletal abnormalities, we characterized a ENU-mutagenesis-derived mouse model with a single mutation in the pore region of TRPV4 protein, V620A (TRPV4V620A), a mutation similar to the human mutation V620I. We analyzed high-resolution micro-computed tomographic (microCT) scans of tibia, spine and intervertebral discs, and developed methods for quantitation of kyphosis and lordosis, in 6-month-old wildtype (TRPV4WT) and TRPV4V620A mice.

TRPV4V620A mice had significantly greater thoracolumbar kyphosis and cervicothoracic lordosis than controls (p=0.008 and p=0.0001, respectively), but no scoliotic abnormalities on the coronal plane. Angles of lumbar lordosis were not significantly different (14.43 ± 1.168° in TRPV4V620A mice, and 19.01 ± 2.516° in TRPV4WT mice, p=0.0765)). Heights of lumbar spine vertebral bodies were not significantly altered, nor was any significant platyspondyly detected. There was no significant change in disc space height or disc wedge angles of lumbar spine between TRPV4WT and TRPV4V620A mice. In the tibia, TRPV4V620A mice had significantly shorter tibial length compared wildtype controls (p=0.0075), but no significant alteration in tibial width (p=0.4667).

These data revealed that the TRPV4V620A mouse shows significant skeletal dysplasia with shorter hindlimbs and hyperkyphosis in the sagittal plane, and suggests this model may be used to provide insight into mechanism by which the human TRPV4 mutation causes kyphosis.
P111
THE CORRELATION BETWEEN INTERLEUKIN-1 BETA AND CLOT KINETICS OF EARLY BONE DEFECT HEMATOMAS
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Aims: It has been revealed that excessive inflammatory responses with an unfavorable impact on bone repair courses are a possible pathomechanism for delayed bone healing. In our previous studies we reported evident differences in the levels of interleukin-1 beta (IL-1β) between hematomas formed in the smaller compared to the larger bone defects. Despite this, few studies concentrated on the direct interplay between the proinflammatory cytokines and structural properties of fracture hematomas.

Methods: The effects of IL-1β on clot kinetics were investigated using Thromboelastography (TEG) at different concentrations (0, 50, and 500 pg/mL). We then evaluated the rigidity of blood clots using compressive studies to measure elastic moduli. The thrombolysis (dissolution) process was assessed by calculating D-dimer amounts.

Results: From the TEG tracing, the significantly prolonged split point, reaction time, and coagulation time of whole blood clot formation with reduced α angle and maximum amplitude were observed in the control group, compared to IL-1β groups (P<0.01). Clot rigidity analysed by compressive studies revealed that 500 pg/mL IL-1β can yield clots with thinner and denser fibres (Fig 1), which was consistent with the outcomes from thrombolytic study showing that the compact fibrin clots contributed to a lower susceptibility to dissolve (P<0.01).

Conclusion: In summary, we can conclude that 500 pg/mL IL-1β can significantly reduce fiber thickness and increase fiber density, altering the mechanical strength and thrombolytic activities of clots. This finding may provide a promising target for augmenting bone regeneration via modulation the quality of clots.

Figure 1: Effect of IL-1β on whole blood clot rigidity
P112
IDENTIFYING RACIAL AND SEXUAL DIMORPHISM IN CORTICAL POROSITY REQUIRES CORRECT POSITIONING OF THE REGIONS OF INTEREST
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Rational: Several studies report higher cortical porosity in men than women, higher cortical thickness and lower cortical porosity in Asians than Caucasians, and thinner more porous cortices in taller than shorter persons [1-3]. The automatically selected ROI in taller persons is more distal than in shorter persons. The ROI is 9.5 mm and 22.5 mm proximal to the mid-joint line for distal radius and tibia, respectively in all individuals regardless of bone length. This may introduce errors in the assessment of cortical and trabecular morphology because of the heterogeneity of the metaphyseal microstructure.

Methods: We imaged the non-dominant distal radius of 80 Caucasian and Asian females and males, age range (25-46 yrs) using HR-pQCT the standard ROI and analysed slice by slice using StrAx 1.0 [4]. The ROI and number of slices were positioned at 4-5.5% forearm length and porosity was quantified comparing the Standard ROI vs ROI Adjusted for length alone and then length and total cross sectional area.

Results: Biologically and statistically significant sex and racial differences in cortical porosity resulted after adjusting the ROI for length and total CSA such that women had higher porosity than men in each race. There was no racial difference in porosity after adjusting for bone length and diameter [Fig 1-3 example of 4 individuals differing by race and sex].

Conclusion: Failure to correctly position the ROI over the anatomical region to be compared in growth, ageing, disease and drug therapy produces misleading information concerning cortical porosity and other morphological traits.

1-Walker, M.D.et al., JBMR 2011
2-Wang, X.F. et al., JBMR 2009
3-Bjørnerem, Å.et al., JBMR 2013
P113
MICROSTRUCTURAL DETERIORATION IN SPINAL CORD INJURY
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Immobilisation is accompanied by bone loss due to a reduction in bone formation and increased bone resorption by osteoclasts. Deficits in bone mineral density (BMD) are well documented in spinal cord injury (SCI) but little data are available quantifying the microstructural abnormalities responsible for the low BMD.

We studied 39 men with complete SCI (44.2 ± 14.5 years, duration of paralysis ranging from 3 weeks to 20 years) and 70 age-matched healthy men recruited from Austin Health, University of Melbourne. Images of the non-dominant distal tibia were obtained using high-resolution quantitative computed tomography (HR-pQCT, Scanco, 82 micron voxel size). Bone microarchitecture and matrix mineralisation density were quantified using StrAx1.0 (StraxCorp, Melbourne, Australia).

Compared to controls, men with SCI had a 43.3% lower cortical area (p<0.05), 51% lower cortical thickness (p<0.001) and 18% higher cortical porosity. Total vBMD was reduced by 25% (p<0.001). Trabeculae were fewer by 45% with a 3.2 fold higher trabecular separation.

Profound and rapid loss of both cortical and trabecular bone underlies the risk for fracture.

Thinner cortex, higher cortical porosity and fewer trabeculae and higher trabecular bone pattern inhomogeneity on the spinal cord injury patient.
MECHANISMS OF PALMITATE-INDUCED LIPOTOXICITY IN OSTEOCYTES
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Apoptosis and autophagy play an important role in the regulation of bone metabolism. The mechanisms triggering these two processes in osteocytes (Ocy) remain poorly understood. Since osteoblasts lose their function and undergo autophagy and apoptosis after exposure to palmitic acid (PA), we then hypothesized that PA has a similar effect on Ocy.

MLO-Y4 Ocy were plated in 0.01% collagen type I-coated plates and cultured in α-MEM in the presence of either PA (250 and 500μM) or vehicle for 48 hrs. Cell viability was measured using MTS-Formazan. Apoptosis was identified by TUNEL and Annexin V. Autophagy was quantified flow cytometry analysis of punctate. Changes in Ocy-secreted proteins (sclerostin [Sost], Dickkopf-related protein 1 [Dkk1] and receptor activator of nuclear factor kappa-B ligand [RANKL]) were determined by western blot.

Treatment with PA induced significantly higher levels of cell death (∼40%, p<0.001) in a time- and dose-dependent manner. PA induced high levels of apoptosis in MLO-Y4 cells (∼35%, p<0.001), with PA-treated cells showing a significantly higher percentage of cytoplasmic area occupied by cytochrome C (p<0.001). In addition, we found a dose-dependent failure in autophagy in PA-treated Ocy (p<0.01). Finally, PA treatment induced higher levels of Sost, Dkk1 and RANKL expression in a dose-dependent manner.

In summary, we have identified a previously unknown effect of PA on Ocy, which could explain the changes observed in Ocy of aged and osteoporotic bone. These findings could play an important role in the understanding of the fat-bone relationship with potential therapeutic applications in the future.

CALCITONIN MODULATES OSTEOCYTE S1P SIGNALING AND SCLEROSTIN PATHWAY EXPRESSION
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Calcitonin (CT) has recently been suggested to have a role as an inhibitor of bone formation. Recently, we demonstrated a potential mechanism by which CT could modulate bone formation, with CT treatment significantly increasing sclerostin expression in bone. More recently, it has been suggested that CT controls bone formation by inhibiting the release of sphingosine 1-phosphate (S1P) from osteoclasts. As we have previously reported that freshly isolated osteocytes express calcitonin receptor (CTR) mRNA this raises the question of what is the action of CT treatment on osteocyte S1P expression.

To resolve this question we used the Ocy454 cells, a novel murine osteocytic cell line. We have previously reported CTR and sclerostin expression are both lost in long-term cultures of osteocytes. Therefore it was necessary to ensure CTR expression in Ocy454 cells. Here, we report that CTR is expressed in Ocy454 cells and increases throughout differentiation. Furthermore, Spkh1 and Spkh2 mRNA, required for intracellular S1P production, Spns2, required for S1P secretion, and the five S1P receptors, S1pr1-5 are expressed at high levels in osteocytes and increase in expression throughout osteocyte differentiation.

Treatment of differentiated (14 days) Ocy454 cells with salmon CT (10nM) for 3 hours significantly increased Spkh1 (1.4 fold, P<0.01) and Spns2 (1.5 fold, P<0.05) mRNA. In addition, Sost mRNA levels were increased (6.5 fold, P<0.01) in Ocy454 cells.

Further studies are ongoing to determine the mechanism of action by which CT modulates S1P and sclerostin signaling, and to determine the dominant effect of CT treatment on bone formation.
CONSTRUCTION OF A NOVEL GENE LIBRARY RELATED TO OSTEOGENIC DISORDER USING EXCHANGEABLE GENE TRAP MUTAGENESIS
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Locomotive syndrome (locomo) is estimated to affect over 47 million people in Japan, with over 12 million of them suffering from osteoporosis, and these numbers are expected to rise amid the aging society. To analyze disease etiology and clarify the pathological mechanisms of osteogenic disorder, we constructed a novel mouse gene library based on bone metabolism screening using an exchangeable gene trap system. The system can simultaneously isolate novel genes and perform functional analysis at an individual level. We isolated trap clones, analyzed trapped genes, and constructed the database for Exchangeable Gene Trap Clones (EGTC) [http://egtc.jp]. A total of 1270 trap clones were identified as present in the database. Mouse lines were established from 490 trapped embryonic stem cell clones. We selected genes with potentially important roles in bone metabolism, and performed bone metabolism screening by micro-computed tomography, bone morphometry, biomechanical strength analysis, X-gal staining, and real-time PCR. Out of 50 lines of homo/hetero mice, 32 were recognized as screening for an abnormality. The Lima1 and Nedd4 knockout mice, for instance, presented bone weakness, while the Tmem161a knockout mouse showed increased bone mass. The bone weakness of the Lbr knockout mouse resulted from a causative gene for Greenberg osteodysplasty. This screening method has the potential to allow thorough and efficient construction of a model mouse library for osteogenic disorder as a cause of osteoporosis. The applicability of the gene-trapping mouse as an effective bioresource for use in various research fields is likely to be of great importance in bone metabolism research.

HISTOCHEMICAL ASSESSMENT ON BONE TISSUES IN TYPE II DIABETIC SDT FATTY RATS
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Introduction: It is known that diabetes mellitus is associated with increased risk of fracture, i.e., diabetic osteoporosis. However, it is still veiled what happens on bone cells in a state of diabetes mellitus. Sprague-Dawley Torii (SDT) fatty rats are good animal models featuring type II diabetes. In this study, therefore, we have examined histological alterations of bone tissues in SDT fatty rats.
Materials and Methods: Forty two male SDT-ffafa (fatty) rats and age-matched SD rats were fixed, and their tibiae and femora were embedded into paraffin and epoxy resin for histochemical examination of ALP, DMP-1, chondroitin-4-sulfate, osteocalcin, CD31, silver impregnation and toluidine blue staining.
Results and Discussion: Unlike the control group, tibial metaphyses of SDT fatty rats developed thin and fragmented trabecules, in which there seemed no active form of osteoblasts bearing an intense ALP-positivity. Silver impregnation revealed well-arranged osteocytic lacunae and their canaliculi in both control and diabetic rats. However, the SDT fatty rats demonstrated a markedly-reduced immunoreactivity of chondroitin-4-sulfate and DMP-1 in the osteocytic canaliculi. However, osteocalcin immunoreactivity was observed in the osteocytes of SDT fatty rats. Semi-thin sections demonstrated an increased number of CD31-positive vascular endothelial cells in the bone marrow region. In our animal model, it seems likely that diabetes mellitus may affect osteocytic lacunae and canaliculi systems and vascular endothelial cells.
**P118**

**DEVELOPMENT OF A NOVEL SUGAR-BASED DELIVERY SYSTEM FOR BONE MORPHOGENETIC PROTEINS (BMPs)**

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**Background:** Recombinant human bone morphogenetic proteins (BMPs) are used in orthopaedics to promote bone repair and new bone formation. The current gold standard for delivery is porous collagen scaffold, but we have explored the application of alternative injectable biomaterials that allow for controlled release and minimally invasive delivery.

**Methods:** A novel compound, pdRAIB, was manufactured via the polycondensation and subsequent esterification of the pro-angiogenic sugar 2-deoxyribose. Biocompatibility and rhBMP-2 delivery studies were performed in a mouse hind-limb implantation model.

**Results:** Biophysical characterization of pdRAIB showed it to be a highly viscous material that could be made injectable by dilution in ethanol. After injection, pdRAIB formed a semi-solid depot that was biocompatible. Delivery of 5µg rhBMP-2 in pdRAIB formed more than 2-fold more bone than the same drug dose in porous collagen. Increased bone and a more regular shape was seen when pdRAIB was loaded in a 3D-printed PLA scaffold, and when combined with the bisphosphonate zoledronic acid. Histology showed evidence of increased inflammation/vascularity in the bone nodules.

**Discussion:** pdRAIB is a versatile biomaterial with multiple potential orthopaedic applications. Future studies will investigate pdRAIB in orthopaedic models, as an implant coating, and for the delivery of alternative agents.

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**P119**

**IN VITRO AND IN VIVO ASSESSMENT OF A POLYHYDROXYBUTYRATE-HYDROXYVALERATE SCAFFOLD FOR BONE TISSUE ENGINEERING**

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Bone tissue engineering requires a biocompatible scaffold that can support cell growth and enhance the native repair process. Polyhydroxybutyrate-hydroxyvalerate (PHB-HV) is a biodegradable scaffold that has previously demonstrated the potential to improve spinal cord repair. This study evaluates PHB-HV as a scaffold for bone regeneration in vitro and in vivo.

Primary human osteoblasts were seeded onto the surface of PHB-HV and cultured for 21 days. Cell viability was assessed using alamarBlue® and Live/Dead® assays. Migration of cells through the scaffold was assessed by DAPI staining and fluorescent imaging across a transverse plane. Alkaline phosphatase activity was measured by p-NPP hydrolysis. For in vivo evaluation, 5-mm critical-sized defects were created in the parietal bone of 40 male adult Sprague-Dawley rats. The rats were sacrificed at 4 or 12 weeks post-operatively and the calvaria imaged by μCT.

Osteoblast numbers significantly increased throughout the culture period on PHB-HV (P<0.05). Fluorescent imaging demonstrated that osteoblasts colonised the surface of PHB-HV and migrated through the porous scaffold. Alkaline phosphatase activity did not alter over time or when compared to osteoblasts cultured on plastic. In vivo, implantation with PHB-HV into rat calvarial defects did not significantly improve bone regeneration when compared to empty defects.

PHB-HV supports the growth of primary bone cells in vitro. However, as an acellular scaffold it does not improve bone regeneration in a calvarial defect. PHB-HV may therefore be better suited as a biocompatible and biodegradable scaffold that can deliver stem cells and/or growth factors to defect sites to encourage bone regeneration.
PROTEASE-ACTIVATED RECEPTOR-2 GENE ABLATION AND THE BONE PHENOTYPE DURING AGEING: A MICRO-CT STUDY
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Protease activated receptor-2 (PAR2), a member of the small family of protease-activated G protein-coupled receptors, is expressed by osteoblasts and osteoclast precursors. During growth, cortical and trabecular bone mass in the tibia is higher in PAR2-knockout mice than in wildtype mice, in association with lower osteoblast and osteoclast surface. The aim of this study was to investigate the effect of PAR2 ablation on bone structure in ageing mice. Littermate PAR2-knockout and wildtype mice were killed at 20 and 32 weeks of age, and tibiae and L5 vertebrae were excised. The trabecular bone of L5 and of the proximal tibial metaphysis, and the cortical bone of the tibial midshaft, were analysed by micro-CT (voxel size 3.4 µm). In tibial and vertebral trabecular bone, BV/TV declined over time in all groups. In males, trabecular BV/TV was significantly smaller in PAR2-knockouts than in wildtypes at 20 weeks, but not at 32 weeks. Trabecular tissue mineral density (TMD) was lower in male knockouts than in wildtypes at both 20 weeks and 32 weeks. In trabecular bone from female mice, however, the only significant difference between genotypes was a lower TMD value for knockouts at 32 weeks. In cortical bone, area fractions were significantly smaller in PAR2-knockout males than in wildtype males; no difference between genotypes was observed in females. However, cortical TMD was found to be lower in PAR2-knockouts than in wildtypes of both sexes. Overall, it was noted that PAR2 plays a role in slowing age-related bone loss in a sex-dependent manner.
**P121**

**ER STRESS TRANSDUCER OASIS MODULATES HYPOXIA PATHWAY ACTIVITY TO REGULATE BONE ANGIOGENESIS**

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OASIS/CREB3L1, an endoplasmic reticulum (ER)-resident transcription factor, plays important roles in osteoblast differentiation. In this study, we identified new crosstalk between OASIS and the hypoxia signaling pathway, which regulates vascularization during bone development. RT-PCR and real-time PCR analyses revealed significant decreases in the expression levels of hypoxia-inducible factor-1α (HIF-1α) target genes such as vascular endothelial growth factor A (VEGFA) in OASIS-deficient (*Oasis-/-*) mouse embryonic fibroblasts. In co-immunoprecipitation experiments, the N-terminal fragment of OASIS (OASIS-N; activated form of OASIS) bound to HIF-1α through the bZIP domain. Luciferase assays showed that OASIS-N promoted the transcription activities of a reporter gene via a hypoxia-response element (HRE). Furthermore, the expression levels of an angiogenic factor *Vegfa* was decreased in *Oasis-/-* osteoblasts. Immunostaining showed retarded vascularization in bone tissue of *Oasis-/-* mice. These results suggest that OASIS affects the expression of HIF-1α target genes through the protein interaction with HIF-1α, and that OASIS-HIF-1α complexes may play essential roles in angiogenesis during bone development.

**P122**

**LOW DOSE CAPE TREATMENT MAY HAVE AN ANABOLIC EFFECT ON GROWTH WHILST IT SUPPRESSES BONE FORMATION IN A CAIA MODEL OF INFLAMMATORY ARTHRITIS**

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Caffeic acid phenethyl ester (CAPE) is an NF-κB inhibitor with anti-inflammatory and immunomodulatory properties.  
**Aim:** To target inflammation and bone loss with CAPE in a collagen antibody induced arthritis (CAIA) model  
**Methods:** 32 Balb/c mice (4 groups of n=8; Control, CAPE, CAIA, CAIA+CAPE). CAPE was administered 1mg/kg/day subcutaneously days 3, 7 and 10. Paws were scored daily for inflammation (max score 16 X 4 paws). Serum was collected on day 14 and measured for CRP and CTX-1 levels (ELISA). Bone volume (BV) was measured in vivo by microCT at baseline and endpoint.  
**Results:** Paw scores were significantly higher in the CAIA+CAPE mice compared with all groups on day 5 (p<0.05). Day 8-14 paw scores did not differ between CAIA and CAIA+CAPE mice but were significantly higher than the control groups (p<0.05), reducing by day 14. CRP levels did not significantly differ between the groups (9.7-10.4ng/ml). CTX-1 levels were highest in CAIA mice (48.1ng/ml) but were not significantly greater than any other group. Though not significant, BV increased more overtime in the CAPE treated control mice whilst BV reduced overtime in CAIA+CAPE mice compared with CAPE alone (p<0.005).  
**Conclusion:** Unexpectedly, CAIA CAPE-treated mice exhibited earlier onset of local inflammation (paw score). Systemic inflammation (CRP-1) and bone resorption (CTX-1) was similar in all groups on day 14. Local inflammation in the CAIA and CAIA+CAPE groups reduced. CAPE may have an anabolic effect on local BV in the physiological environment whilst unexpectedly it suppressed bone growth in the presence of inflammation.
THE ROLES OF CSPG4 AND PERLECAN IN CANCER, POTENTIAL TARGETS FOR DRUG THERAPY
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Metastasis is characterised by the spread of cells from a primary cancer to distant organs and tissues. This relies on changes in cell-cell and cell-extracellular matrix (ECM) interactions. Previous studies have shown that the proteoglycans CSPG4 and perlecan, are able to mediate some of these cell-ECM interactions, identifying them as potential immunotherapeutic targets to control cancer spread. The study aims to identify the structures of CSPG4 and perlecan produced by colon carcinoma (WiDr) and melanoma (MM200 and Me1007) cell lines and to analyse their interactions with the ECM components.

The structural analyses showed that the CSPG4 and perlecan expressed by MM200, Me1007 and WiDr cell lines were structurally different. The structural differences were not only in the protein core, but also in the GAGs. CSPG4 and perlecan were found to interact with collagens types I, IV, V and VI. The roles of CS in CSPG4, and CS and HS in perlecan, in interacting with collagens were different between cell lines. This indicated that the GAGs structural differences resulted in different interactions with the ECM molecules. A novel finding in this study is that CSPG4 was demonstrated to interact with perlecan. Further investigation also showed that CSPG4 interacted with the C-terminal domain of perlecan.

In conclusion, the GAG structures decorating CSPG4 and perlecan are cell line dependent, which explain the binding differences observed between CSPG4 and perlecan from different cell lines and collagens. The interaction between CSPG4 and perlecan could be implicated in the cross-talk between tumour-host compartments.

SPINAL BONE DUST IS A SIMPLE AND EFFECTIVE BONE DUST MATERIAL
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Introduction: In spinal fusion surgery, bone grafting is routinely performed to encourage osseous union. Autologous iliac crest bone is the ‘gold standard’ graft choice, however, significant surgical complications have highlighted the need to develop alternative graft sources. Bone dust particulates generated during spinal surgery are usually lost via suction, yet are a simple graft alternative to iliac crest bone. Previous studies have demonstrated that bone dust is a source of viable osteoblasts. Here, we hypothesised that bone dust is also a source of anabolic factors.

Aim: To assess the osteoinductive potential of bone dust harvested during spinal fusion surgeries.

Methods: Bone dust was collected using a suction trap and transferred to 1μm pore-size tissue culture inserts suspended over primary human osteoblasts cultured in 24-well plates. The effect of bone dust on osteoblast proliferation, matrix production and gene expression was assessed over 7 days by 3H-thymidine incorporation, Sirius red staining and real-time PCR, respectively. Release of alkaline phosphatase from bone dust was determined by p-NPP hydrolysis.

Results: Osteoblast proliferation increased ~5-fold and collagen production increased by 19% in the presence of bone dust compared to control (p<0.0001 for both). There was no difference in expression of osteoblastic genes between groups, however, inflammatory cytokine and angiogenic genes were upregulated in bone dust cultures. Alkaline phosphatase was released in high concentrations from the bone dust.

Conclusions: Bone dust releases factors that are anabolic to bone. These results suggest that bone dust has therapeutic potential to be used as a simple and effective autograft.
P125
USE OF THE GENE MINE MOUSE PHENOTYPE LIBRARY TO IDENTIFY NOVEL GENES REGULATING BONE MASS
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It is well established that there is a strong genetic effect on bone mass, bone loss and fracture rates; however, the vast majority of genetic variance for osteoporosis-related phenotypes remains unexplained. We have utilised a novel mouse resource, the Gene Mine, to identify genes associated with changes in bone mass in mice. µCT was used to analyse several bone parameters and the mice were then stratified based on individual parameters to allow for identification of genetic variations related to bone mass and architecture. We then correlated these genes with human genetic variation and osteoporosis risk. To date we have only screened a single bone parameter, trabecular bone mass. Our analysis identified several genes associated with trabecular bone mass in mice. When these genes were correlated with human genetic data from osteoporosis datasets a significant association was found among variation in Thioredoxin interacting protein (Txnip), BMD and fracture risk in humans. This protein has not been previously associated with bone mass, but functions in multiple pathways governing oxidative stress, inflammation, and angiogenesis, all of which are known regulators of bone homeostasis. The success of our preliminary screening process has shown it is feasible to utilise the Gene Mine mouse resource for gene discovery in mice and humans. We are now extending our analysis to additional bone parameters, including measures of bone mass and bone architecture in both the axial and appendicular skeleton. This approach promises to generate significant new genetic targets for the development of osteoporosis treatments.

P126
VANIN-3 SIGNALLING IN OSTEOARTHRITIS
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We recently discovered that the most highly upregulated gene in mouse cartilage explants following stimulation with IL-1α, was the little-known, vanin-3. Our preliminary experiments in mice have confirmed that vanin-3 is expressed in cartilage explants, cultured chondrocytes, synovial fibroblasts and macrophages, and that its expression is upregulated by inflammatory cytokines. Although vanin-1 is a pantetheinase that hydrolyses pantetheine to vitamin B5 and cysteamine, vanin-3 lacks pantetheinase activity, and is instead thought to be a key component of Toll-like receptor (TLR) signalling. We are investigating the role(s) of vanin-3 in modulating cartilage pathology in experimental OA and exploring the link between vanin-3, OA and metabolic disease. We have found that vanin3 is highly upregulated by the pro-inflammatory 32mer peptide derived from aggreganolysis, and that upregulation of vanin3 by IL-1 and 32mer is dependent upon MyD88 and TLR2 signalling. In the absence of a challenge, vanin-3 null mice are healthy, viable and fertile.
**P127 IN VITRO ISOSORBIDE MONONITRATE DOES NOT AFFECT OSTEOBLAST GROWTH AND DIFFERENTIATION IN THE ABSENCE OF NO RELEASE**

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**Background:** Nitrates (including nitroglycerin (NTG) and isosorbide mononitrate (ISMO)) are under investigation for prevention of bone loss. *In vivo*, markers of bone formation are increased and markers of bone resorption are decreased, accompanied by prevention of bone loss or increase in bone density. In a single *in vitro* study NTG increased proliferation and osteoblast differentiation in human bone marrow mesenchymal stem cells. Here we investigate *in vitro* effects of ISMO in osteoblastic or stromal cells.

**Methods:** The effects of ISMO and a spontaneous nitric oxide (NO) donor (DETA-NONOate) were evaluated in murine stromal (ST2) and osteoblastic (MC3T3-E1) cell lines and in primary rat (RObs) and human osteoblasts (HObs) using [3H]-thymidine mitogenesis assays and real-time gene expression. NO release was measured in conditioned media using the Griess assay.

**Results:** ISMO (0.01-1000ug/mL) did not affect mitogenesis or gene expression associated with osteoblast differentiation, however minimal NO release was detected. DETA-NONOate (10-100uM) inconsistently stimulated mitogenesis and increased expression of genes associated with differentiation in HObs. In ST2 cells and RObS, DETA-NONOate had the opposite effect. There was a dose-dependent release of NO.

**Conclusions:** These results suggest that *in vitro*, ISMO has no effect on stromal and osteoblastic cells in the absence of detectable NO release. In the presence of NO release, DETA-NONOate affected osteoblast growth and differentiation in human and rodent cells. Further study is indicated to determine reasons for these negative findings *in vitro* and to clarify the role of NO in mediating effects of nitrates on bone density and metabolism.

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**P128 CUMAMBRIN A INHIBITS OSTEOCLASTOGENESIS AND OVARIECTOMY-INDUCED OSTEOPOROSIS VIA SUPPRESSION OF NF-KB AND NFAT SIGNALLING PATHWAYS**

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Osteoclasts are the principal cells responsible for bone resorption and pathological bone loss. So, they are the main targets of anti-resorptive therapy. The aim of this project is to investigate the therapeutic potential and molecular mechanisms of novel natural compounds on osteoporosis. We studied the effect of cumambrin A on cell viability using BMM. The effect of cumambrin A on the expression of osteoclast maker gene was tested by RT-PCR. The effect of cumambrin A on the RANKL-induced signal pathway was determined by western blot and luciferase reporter gene assays. The effect of cumambrin A on the activation of osteoclast was examined by bone resorption assay. The O VX mouse experiment was performed to test the effect of cumambrin A on estrogen deficiency-induced bone loss.

We found that cumambrin A inhibits the expression of osteoclast marker genes, including cathepsin K, calcitonin receptor, and V-ATPase d2. Cumambrin A can significantly inhibit the RANKL-induced osteoclasts and bone resorption through the suppression of NF-kB and NFAT activity. In addition, cumambrin A can also inhibit RANKL-induced ERK phosphorylation. This concentration of cumambrin A (5µM) didn’t affect the viability of osteoclasts precursor cells. *In vivo* study revealed that cumambrin A reverse the bone loss induced by ovariectomy. Collectively, our results show that cumambrin A suppresses the RANKL-induced osteoclasts and bone resorption, suggesting cumambrin A may be a potential treatment of bone destruction related disease.
A NEW MODEL TO STUDY THE IMMUNOREGULATORY EFFECT OF EXOGENOUS FACTORS ON MACROPHAGES
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Introduction: Macrophages derived from hemopoietic progenitors and circulating monocytes perform an important role in homeostasis and regeneration. Classically activated macrophages (M1) develop in response to interferon-gamma (IFN-γ) and microbial stimuli such as lipopolysaccharide (LPS), which activate toll-like receptor-4 (TLR-4) signaling in macrophages. Alternatively activated macrophages (M2) in vitro are controlled by interleukin-4 (IL-4) and interleukin-13 (IL-13). Because macrophages have often been studied in the context of host defense, their activation responses have been closely associated with pathogenic stimuli. Therefore, the traditional way to study macrophages is normally based on pathogen stimulation. However, macrophages were still found in some typical situations such as bone forming and bone remodeling during which macrophages were rarely activated by endogenous or exogenous pathogens. These observations implicate that macrophages may be a more multifunctional group of cells than originally appreciated, with different physiologies and performing distinct immunological functions. This study aims to investigate the immunoregulatory effect of exogenous factors on macrophages under physiological rather than pathological conditions to find out a novel approach for macrophage studies during bone remodeling.

Methods: A murine macrophage cell line RAW264.7 was cultured in normal growth medium supplemented with vascular endothelial growth factor-A (VEGF-A) and bone morphogenetic protein-2 (BMP-2) as the exogenous stimuli. Proinflammatory cytokines secreted by macrophages was examined by RT-PCR and ELISA. The morphological changes of macrophages were examined by phalloidin staining. M1 phenotype of macrophages was stimulated with LPS and IFN-γ and M2 phenotype was stimulated with IL-4 supplemented by VEGF and BMP-2 respectively. M1 and M2 related genes were analyzed by RT-PCR. The expression of iNOS and arginase were detected by immune fluorescence.

Results and Discussion: Morphological changes were observed in macrophages induced with VEGF and BMP-2 with or without LPS+IFN-γ or IL-4 stimulation (Figure 1). Our data has demonstrated that M1 markers such as inducible nitric oxide synthase (iNOS), C-C chemokine receptor type 7 (CCR7), cluster of differentiation 86 (CD86) and integrin alpha X (ITGAX, CD11c) were up-regulated by VEGF and down-regulated by BMP-2 with or without LPS and IFN-γ. M2 markers such as Arginase, interleukin-10 (IL-10), cluster of differentiation 163 (CD163), cluster of differentiation 206 (CD206) were up-regulated by BMP-2 and down-regulated by VEGF with or without IL-4.

Figure 1: The morphological changes of macrophages induced with VEGF and BMP-2 with or without LPS+IFN-γ or IL-4.

Conclusions: Our results indicate that exogenous stimuli were able to slightly activated macrophage polarization without LPS+IFN-γ or IL-4, which is closer to the bone homeostasis. The interplay between exogenous factors and macrophages under non-inflammatory conditions should be elucidated when evaluating the in vitro osteogenic capacity of target proteins and biomaterials.


Aim: Bone erosion is common in advanced gout. In erosive gout, monosodium urate (MSU) crystals are observed in subchondral bone adjacent to osteocytes. Given that osteocytes are important regulators of bone remodelling, this study aimed to investigate the effects of MSU crystals on osteocyte viability and gene expression.

Methods: MSU crystals (0.01 -0.5mg/mL) were added to MLO-Y4 osteocyte-like cells or primary mouse osteocytes (isolated from long bones) cultured in type I collagen gels. After 24h, MSU crystals were completely removed. Cell viability was assessed both 24h and 48h after the addition of MSU crystals using alamarBlue assays. Soluble urate and other types of crystals (basic calcium phosphate, calcium pyrophosphate and aluminum) were also tested in MLO-Y4 cultures using the same method. Real-time PCR was used to examine changes in osteocyte-related gene expression in MLO-Y4 cells cultured as above with 0.1mg/mL MSU crystals for 6h or 24h.

Results: In the viability assays, at the 24h timepoint, higher concentrations (0.3 and 0.5mg/mL) of MSU crystals reduced both MLO-Y4 cell (Figure) and primary osteocyte viability by ~30-40%. At the 48h timepoint, there was further reduction of viability by ~60-70%. The effect on cell viability was specific to MSU crystals, as soluble urate and other crystal types did not reduce MLO-Y4 viability. Culture with MSU crystals did not alter gene expression of connexin43, E11, ORP150, osteocalcin, RANKL or OPG in MLO-Y4 cells.

Conclusions: MSU crystals inhibit osteocyte viability, but do not affect osteocyte-related gene expression. Reduced osteocyte viability may contribute to bone erosion in gout.

Figure: MSU crystals reduce the viability of MLO-Y4 osteocyte-like cells in type I collagen gel cultures. Viability was assessed using the alamarBlue assay 24 and 48 hours after the addition of MSU crystals. Data are pooled from three biological repeats and are presented as mean (SEM); two-way ANOVA (P<0.0001) with post hoc Dunnett’s test *p<0.01, **p<0.001 versus control (no MSU crystals) for the relevant timepoint.
**Scientific Program**

**Poster Presentations – Basic Science**

**P131**

**ADVERSE EFFECTS OF HIGH GLUCOSE ON BONE AND MUSCLE CELLS IN VITRO**

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Increased bone fragility and reduced skeletal muscle quality are complications of long-term hyperglycemia in type 2 diabetes mellitus (T2DM). T2DM patients have reduced bone turnover markers, indicating bone cells are adversely affected. We showed even short-term hyperglycemia results in reduced bone turnover markers. Skeletal muscle is also adversely affected by long-term hyperglycemia with loss of mass and strength. Human diabetic muscle showed increased MAFbx/Atrogin-1 and MuRF1 activity, indicating upregulation of the ubiquitin proteasome pathway, and increased centralized nuclei, indicating muscle damage. We demonstrated high glucose concentrations in vitro promote apoptosis, reduce viability and ALP, in primary human osteoblasts (HOBs).

Aim: To determine molecular pathways involved in these adverse effects on bone and muscle cells. HOBs and differentiated C2C12 myotubes were maintained in 5mM glucose media for 2 weeks before treatment with increasing doses of D-glucose for 7, 14 or 25 days. Optimal glucose for mineralizing cultures of HOBs was 5mM (Alizarin red). Incubation in 60uU/ml insulin improved mineralization scores in all tested glucose concentrations. Immunoblot analysis of 25d cultures showed high glucose reduced BMP7 and phosphorylation of Smad1/5(serine463/465), the BMP7 downstream target. Other BMPs and the Erk1/2 pathway were not affected. Sclerostin, a negative regulator of bone, was increased with high glucose. In myotubes exposed to 14d 20mM glucose, bioenergetic function (respiration and glycolysis) was reduced to levels comparable to no glucose (Seahorse Flux (XF)). These findings reveal novel pathways involved in musculoskeletal complications observed in patients with long-term hyperglycemia.

**P132**

**THE EXOSOMES FROM ADIPOSE TISSUE- DERIVED MESENCHYMAL STEM CELLS: POTENTIAL APPLICATION FOR BONE TISSUE REGENERATION**

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The University of Sydney

Mesenchymal stem cells (MSCs) are a promising cell source for tissue repair and regeneration. However, direct MSCs transplantation to tissue injury sites has its inherent drawbacks such as senescence-induced genetic instability and limited cell survival. The aim of this study was to employ the exosomes produced by MSCs for use in bone tissue regeneration. We demonstrated that adipose tissue-derived MSC (ASCs)-derived exosomes (ASC-EXO) promoted the proliferation, mobilization and osteogenic differentiation of human primary osteoblastic cells (HOBs). We further established that the trophic effects of ASC-EXO on HOBs were potentiated when ASCs were pre-conditioned with tumor necrosis factor-alpha (TNF-α) for 3 days, mimicking the inflammatory phase of bone fracture healing. In addition, we showed that TNF-α pre-conditioning significantly increased the Wnt-3a content ASC-EXO, and blocking Wnt signaling inhibited the osteogenic gene expression levels in HOBs cultured in the conditioned medium collected from TNF-α pre-conditioned ASCs. In summary, this study demonstrates that ASC-derived exosomes are capable of promoting proliferation, migration, and osteogenic differentiation in HOBs, and the effects are further harnessed by TNF-α pre-conditioning through wnt signaling pathway, suggesting that ASC-derived exosomes might offer a promising approach to replace direct stem cell transplantation for bone repair and regeneration.
**P133**

**IN VITRO IMMUNOGENICITY SCREENING OF TWO NOVEL BONE GRAFT SCAFFOLDS**

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**Background:** Bone grafts are used when bone loss exceeds the body’s healing capacity. However, currently used grafts have unacceptable complication rates (≈50%). Many potential graft alternatives (scaffolds) are rushed through to clinic, and ultimately fail. Immune rejection is often implicated, creating substantial need for understanding how novel scaffolds interact with the immune system.

**Aim:** To screen novel scaffolds in immune cell assays in vitro, enabling predictions of immune response.

**Methods:** Human macrophage-like THP-1 cells were exposed to two novel PLA and PETG scaffolds. Gene expression and protein secretion levels of inflammatory cytokines were assessed at 1, 3 and 7 days post-culture. VICRYL® deep-tissue suture material was used as a clinical control.

**Results:** PLA and PETG scaffolds increased IL-1β gene expression >800 and >275-fold respectively, on day 1 compared to negative control (p<0.0001), and remained high throughout the culture period (day 3; p<0.01). Other pro-inflammatory cytokine expression, and IL-1β protein secretion, mimicked IL-1β gene expression over all time-points. Interestingly, IL-1β expression was significantly higher for VICRYL® when compared to negative control cultures on day 1 (p=0.002).

**Conclusion:** Screening indicated both scaffolds, that were due for in vivo investigation, would likely result in immune rejection. When PLA and PETG polymers were evaluated alone, cytokine expression was lower, suggesting the observed response is scaffold-, not material-specific. Endotoxin testing is planned to rule out contamination. Overall, this pre-clinical screen identified unsuitable biomaterials, preventing unnecessary in vivo studies. Immunogenicity screening can reduce animal usage and ensure safer transition of novel bone scaffolds through to clinic.

**P134**

**MODULATION OF AUTOPHAGY AND APOPTOSIS IN HUMAN OSTEOCLASTS IN VITRO**

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**Aim:** The aim of this study was to investigate the effect of autophagy and apoptosis modulators on osteoclastogenesis and bone resorption in vitro.

**Methods:** Osteoclasts were differentiated in vitro from human peripheral blood monocytes in the presence of M-CSF and RANKL. Cells were pretreated with TNF alpha (5ng/ml) for 24 hours before treatments. Autophagy inhibitor (Hydroxychloroquine/HCQ 50μg/ml), autophagy inducer (Rapamycin 100μM) and apoptotic inducer (Embelin 15μmol/l) were administered for 24 hours. Effect of modulators was investigated using quantitative real-time PCR and immunofluorescent staining of autophagy genes (Beclin-1, LC3), apoptotic markers (caspase 3 and 9) and TUNEL. Osteoclast formation and function were investigated using tartrate resistant acid phosphatase (TRAP) and dentine resorption assay respectively. Effect of treatments was also visualised using live cell imaging and transmission electron microscope.

**Results:** HCQ and Embelin suppressed Beclin-1 at 6 hrs and LC3 mRNA at 24 hrs. Embelin induced caspase-9 at 6 hrs while HCQ induced caspase-3 mRNA at 24 hrs. Beclin-1 ad LC3 protein, TRAP count, dentine resorption and cell size were reduced following HCQ and Embelin treatments. The reduced cell number by HCQ and Embelin was associated with increased TUNEL positive cells. Live cell imaging and TEM support the evident of apoptosis following HCQ and Embelin treatment.

**Conclusion:** These findings support that pharmacological modulation of autophagy is associated with changes in apoptosis genes and vice versa and these modulations affect osteoclast formation and function.

**Acknowledgement**

This study is supported by Grant in Aid 2014, the Arthritis Australia, State and Territory Affiliates, Arthritis SA
P135

ESTABLISHMENT AND CHARACTERIZATION OF C2C12 CELLS EXPRESSING HUMAN ALK2 UNDER THE CONTROL OF TET-ON SYSTEM

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Fibrodysplasia ossificans progressiva (FOP) is a rare autosomal dominant disorder characterized by progressive heterotopic ossification in soft tissue such as skeletal muscle. In patients with FOP, ALK2, a type I receptor of bone morphogenetic proteins (BMPs), has been activated by several types of substitution mutations in the intracellular domain. We and others have shown that those mutant ALK2 associated to FOP activated BMP signaling in C2C12 myoblasts. In the present study, we established and characterized subclones of the C2C12 cells that express human ALK2 under the control of Tet-On system. Firstly, we introduced an rtTA-expression vector (pTet-On-Advanced) into C2C12 cells and selected a clone C216, which showed the highest induction of a luciferase reporter activity in the presence of doxycycline (Dox). Next, we constructed Dox-inducible-expression vectors (pTRE-Tight-ALK2) carrying V5-tagged wild-type or mutant human ALK2(R206H). We stably introduced those expression vectors into C216 cell to establish inducible ALK2-expressing cell lines. A Dox-dependent expression of human ALK2-V5 was confirmed by FACS, Western blot and immunohistochemical analyses in both types of subclonal cells. An activity of BMP-specific luciferase reporter was induced by adding Dox without BMPs in Tet-On-ALK2(R206H) cell lines, but not in any Tet-On-ALK2(WT) cell lines. Moreover, an ALP activity induced by BMP-treatment, a typical marker of osteoblastic differentiation, was synergistically increased by the presence of Dox in Tet-On-ALK2(R206H) cell lines. Taken together, these data suggest that our Tet-On-ALK2 cell lines derived from C2C12 myoblasts are useful tools for studying molecular mechanisms of heterotopic bone formation in FOP.

P136

BIOMECHANICAL CHARACTERISATION OF TENDON TISSUE USED AS AUTOGRフト IN RECONSTRUCTIVE KNEE SURGERIES: WHAT CAUSES THE HIGH FAILURE RATE IN YOUNGER PATIENTS?

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Background: Anterior cruciate ligament (ACL) tears are the most common ligamentous knee injuries and costs New Zealand ≈$18,250,000 per annum. Arthroscopic surgical reconstruction using hamstring autograft is the common treatment option, however, there are re-rupture risks. A UniSports Clinic (Auckland, NZ) study of 694 patients showed that those under 20yrs old using hamstring autograft were three times more likely to re-rupture - 10.4% compared to 3.6% (global rate).

Aim: To determine the underlying reasons for the high re-rupture rates of young patients after ACL reconstruction using hamstring autograft.

Methods: During ACL reconstruction, hamstring tendons were harvested and the sections not used as a graft were collected for our study. Tendons were divided into sections for histology, gene expression and biomechanical testing. The biomechanical testing of 18 hamstrings was carried out on an Instron 5800, with stiffness, modulus and ultimate load-to-failure compared between patients under the age of 20 and those over.

Results: There were no statistical differences in hamstring mechanical properties between the two age groups – 18.9 N/mm and 14.6 N/mm for under and over 20, respectively. However, a distinct group of younger patients had a stiffness of over 20 N/mm, while the majority of patients were under 15 N/mm.

Conclusions: A group of patients have been identified with stiffer tendons than the majority, which could be a factor leading to higher re-rupture rates for younger patients. We are following the cohort and will collect re-rupture rates and match it to all tendon parameters (histology, gene expression and biomechanical results).
P137
THE DEVELOPMENT OF BONE IN MPS MOUSE MODELS
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Patients with mucopolysaccharidosis (MPS) accumulate glycosaminoglycans in multiple tissues. Short stature and dysostosis multiplex are common symptoms observed in 6/11 of MPS. The mechanism underlying bone disease in MPS is not fully understood. Here, we investigated the histological abnormalities in bones of different MPS mouse models, aiming to understand the bone pathology of MPS.

MPS I, MPS IIIA, severe MPS VII, attenuated MPS VII and MPS IX femur, tibia and L5 vertebrae were harvested and bone length was determined from radiographs. Femur, tibia and vertebrae in severe MPS VII mice were severely shortened, reaching 67%, 81% and 86% of normal at maturity, respectively. Likewise, the length of femur, tibia and vertebrae were also reduced in attenuated MPS VII mice to approximately 92%, 96% and 95% of normal. MPS I femur but not the other bones was shortened to 95% of normal. Other MPS mice showed no pronounced difference in bone length to normal. Consecutive tibia sections were stained with Safranin O to investigate the formation of bone in MPS and normal mice from 15.5dpc to 2months of age. Formation of the primary and secondary ossification centres were severely delayed in MPS VII mice, concomitantly with anomalous resting, proliferative and hypertrophic regions in the growth plate. Our study highlights the importance of MPS VII mouse model in understanding bone pathology of MPS, and presents evidence for the delayed conversion from cartilage to bone in MPS bone. Ongoing studies seek to determine signalling pathways causing the dysfunction of bone formation in MPS.

P138
IMMUNOLOCALISATION OF INTER-α TRYPSIN INHIBITOR IN HUMAN CARTILAGE AND STRUCTURAL CHANGES THAT MODIFY ITS FUNCTION
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1UNSW, 2University of Sydney and UNSW, 3Royal Prince Alfred Hospital

Inter-α-trypsin inhibitor (IαI) is composed of heavy chains (HC) 1 and 2 that are covalently attached to the chondroitin sulphate (CS) chain of bikunin. IαI is noted for its ability to modify hyaluronan (HA) through the transesterification of its HCs onto HA in the presence of TSG-6. This process is known to be elevated in arthritis, however has not been explored in cartilage development. In this study we examined the localisation of IαI in developing human cartilage and human osteoarthritic cartilage and structurally characterised IαI from osteoarthritis (OA) and rheumatoid arthritis (RA) patients. IαI was immunolocalised to the terminally differentiating chondrocytes in developing human cartilage. In human OA cartilage, IαI was immunolocalised to the lacunae surrounding the chondrocytes in all zones of the cartilage even though fewer chondrocytes were found in the fibrillated surface and superficial zones than the deep zone. IαI was isolated from urine, plasma and synovial fluid samples using anion-exchange chromatography. Plasma- and synovial fluid-derived IαI, together with urinary bikunin isolated from OA and RA patients, contained CS that was lower in sulphation levels compared with plasma IαI and urinary bikunin CS from control subjects. The IαI that contained the more highly sulfated CS chain was found to be more effective at promoting the formation of HC-HA complexes resulting in the stabilisation of HA in the cartilage matrix. This suggests that the low sulfated forms of IαI in OA and RA patients have a reduced ability to form HC-HA complexes and stabilise HA in the cartilage.
P139
MATRIX-CAPTURING SYNTHETIC HYDROGELS FOR DIRECTED TISSUE GENESIS
Hezaveh Hadi, Cosson Steffen, Otte Ellen, Su Guannan, Fairbanks Benjamin, Cooper-White Justin
CSIRO

Extracellular matrix (ECM) molecules play a crucial role in determining stem cell fate choices, such as adhesion, migration, proliferation and even differentiation. Adding ECM proteins into synthetic biomaterials have been previously used in an attempt to direct stem cells to a specific lineage. However, current hydrogel systems are unable to exploit all of the remodelling characteristics of the cell secretome, excepting their ability to degrade protein/polymer chains throughout the hydrogels. The aim of this work is to introduce into a PEG-based system a protein binding peptide (PBP) that enables specific recruitment of cell-secreted proteins. We hypothesise that the ability to retain secreted proteins within the gel will allow cells to begin to form a tissue matrix, replacing the gel as it degrades.

To achieve this, a PBP was synthesized, characterised and its binding affinity to the target protein validated using quartz crystal microbalance with dissipation (QCM-D). A functionalized PEG-based hydrogel was also synthesized and then conjugated with the PBP using click chemistry. Rheology tests proved that the gels possess a wide range of mechanical properties that can be tailored by varying UV exposure time, monomer and cross-linker concentrations. The observed degradation implies that the gels are useful for a range of cell culture purposes. Finally, the ability of gels to recruit secreted protein from various cell types, including mesenchymal stem cells (MSCs), was investigated. In this work, we have introduced a PEG-based system of tunable mechanics, capable of recruiting specific cell-secreted proteins, that offers the opportunity to closer mimic the matrix dynamics of *in vivo* environments for encapsulated cells.

P140
UNLOADING OF SUBCHONDRAL BONE THROUGH LOSS OF ARTICULAR SURFACE CONGRUITY RESULTS IN FOCAL BONE RESORPTION.
Thomas Megan1, Trope Gareth2, MacKie Eleanor1, Whitton R. Chris1
Sciences, Charles Sturt University

A high load environment inhibits bone remodelling activity yet focal remodelling is associated with areas of damage in subchondral bone of athletic horses in training. Focal offsetting of remodelling inhibition may be due to targeting of damaged areas by BMUs or local unloading of subchondral bone due to loss of articular surface congruity. To test whether local unloading of the articular surface due to loss of articular surface congruity can stimulate a focal bone remodelling response we created an osteochondral defect (approximately 15 x 10mm) in the distal facet of the radial carpal bone of six adult horses. Two weeks post-operatively the animals began an eight-week treadmill training program, following which they were euthanased and osteochondral wedges cut from the articular surface opposing the lesion. Control samples were collected from the contralateral limb. The osteochondral wedges were imaged with microCT and cryosections of decalcified specimens stained for TRAP to identify osteoclasts. Bone volume fraction was lower in treated joints 3-6mm below the cartilage in the third carpal bones opposing the defect (n=6, mean difference 0.10, 95% CI (0.02, 0.18), \(P = 0.02\)). Osteoclast numbers were greater in treated joints in bone 0-3 mm below the cartilage at the same site (n=3, mean difference 11 cells/mm² (6, 15), \(P = 0.01\)). Unloading of subchondral bone due to loss of articular surface joint congruity stimulates focal bone resorption. This demonstrates a mechanism by which focal subchondral bone remodelling may occur at sites of subchondral bone damage in a high load environment.
P141
LOWER LIMB FRACTURES AT A REGIONAL UNIVERSITY HOSPITAL
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School of Medicine, Deakin University, 285 Ryrie Street, Geelong, Vic 3220, Australia

Background/Aim: Approximately 1 in 3 women aged ≥50 years will sustain an osteoporotic fracture during their lifetime. Hip fractures are the most severe osteoporotic fracture, but other fractures of the lower limb are also associated with high public health costs, disability and lower quality of life. The aim of this study was to understand the epidemiology and burden of lower limb fractures.

Methods: Incident fractures of the hip, femur, tibia/fibula, ankle and foot in women aged ≥20 years were ascertained from 01/01/2014 to 31/12/2014 using radiology reports at the University Hospital Geelong. Medical records were used to determine hospitalisations.

Results: During 2014, there were 587 fractures of the lower limb in women, including 209 hip, 44 femur, 41 tibia/fibula, 162 ankle and 131 foot fractures. Most fractures of the lower limbs occurred in older women; median ages (IQR) were 85.3 (78.3-90.2) for hip, 81.6 (64.5-88.2) for femur, 62.3 (53.5-81.9) for tibia/fibula and 58.7 (43.9-71.5) for ankle fractures. Fractures of the foot peaked at 50-69 years of age, with fewer fractures occurring in the younger and older age groups (median age 55.1 year; IQR 41.0-65.8). The percentages of women who were hospitalised after sustaining hip, femur, tibia/fibula, ankle and foot fractures were 94.7, 79.5, 56.1, 54.9 and 12.2, respectively.

Conclusion: Fractures of the lower limb occurred commonly in older women. As with hip fractures, most were hospitalised with the exception of foot fractures.

P142
SPONTANEOUSLY OCCURRING KNEE CARTILAGE SURFACE SCORES IN AGEING SHEEP
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1University of Auckland, 2Massey University

Accounts of naturally occurring osteoarthritis (OA) in sheep are rare. Here, we report articular cartilage findings in the stifle (knee) joint of 6-year-old sheep (n = 40). Articular cartilage surface of patella, proximal tibia, femoral condyles (FC), lateral and medial trochlear ridge (LTR, MTR) and trochlear groove (TG) were examined under bright light, stained with India Ink and classified using a modified OARSI system.

Obvious defects in the meniscus were not detected, and there was no evidence of inflammation in adjacent tissues. Cartilage surface scores of 13 different regions are shown (Table 1). There were marked differences between scores in tibial and femoral axial and abaxial sites, which coincided with non-meniscal and meniscal areas. Fibrillation, matrix loss and linear fissures were observed on the cartilage surface.

There was no history of lameness, and no obvious changes in adjacent tissues; defects were reasonably mild, and how long they had been present was not known. There was no signs of inflammation or changes in altered loading, which is more commonly observed in surgical models of experimental OA. The defects appeared to be naturally occurring, similar to a recent study by Vanderweerd et al, and differed in severity and localisation to previously reported surgical models. This may represent early features of spontaneously-occurring ovine OA and could provide a more accurate model of idiopathic human OA. Study of such lesions, their time of onset, and rate of progression may be a useful tool in understanding OA pathogenesis associated with aging.


Table 1: Femoro-patellar-tibial hyaline cartilage surface scores (mean ±SD) of aged sheep.

<table>
<thead>
<tr>
<th>Patella</th>
<th>Tibia Lateral</th>
<th>Tibia Medial</th>
<th>FC Lateral</th>
<th>FC Medial</th>
<th>Troclea</th>
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<td>Prox</td>
<td>Distal</td>
<td>Abax</td>
<td>Abax</td>
<td>Abax</td>
<td>LTR</td>
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<td>2.3±1.8</td>
<td>4.6±1.9</td>
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<td>4.3±2</td>
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<td>0.6±1.0</td>
<td>0.7±0.6</td>
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GLUCOSE-LOADING REDUCES BONE REMODELLING IN WOMEN AND OSTEOBLAST FUNCTION IN VITRO
Itamar Levinger, Ego Seeman, George Jerums, Glenn K McConell, Mark S Rybchyn, Samantha Cassar, Elizabeth Byrnes, Steve Selig, Rebecca Mason, Peter R Ebeling, Tara C Brennan-Speranza

Background: Ageing is associated with a reduction in osteoblast life-span and the volume of bone formed by each basic multicellular unit (BMU). Each time bone is resorbed, less is deposited producing microstructural deterioration. Ageing is also associated with insulin resistance and hyperglycaemia, either of which may cause, or be the result of, a decline in undercarboxylated osteocalcin (ucOC), a protein produced by osteoblasts that increases insulin sensitivity. We examined whether glucose loading reduces bone remodelling and ucOC in vivo and osteoblast function in vitro, and so compromises bone formation.

Methods: We administered an oral glucose tolerance test (OGTT) to 18 pre- and post-menopausal, non-diabetic women at rest and following exercise and measured serum levels of bone remodelling markers (BRMs) and ucOC. We also assessed whether increasing glucose concentrations with or without insulin reduced survival and activity of cultured human osteoblasts.

Results: Glucose-loading at rest and following exercise reduced BRMs in pre- and post-menopausal women and reduced ucOC in postmenopausal women. D-glucose (≥10mmol/L) increased osteoblast apoptosis and reduced cell activity compared with 5 mmol/L. Insulin had a protective effect on these parameters.

Conclusions: Circulating glucose reduces osteoblast viability, at least in culture. Suppression of remodelling may preserve bone mineral density by slowing bone loss, but sacrifice bone’s material composition. The failure of exercise to attenuate the suppressive effect of glucose on remodelling markers may be due to a direct detrimental effect of glucose on osteoblast survival and function.

GESTATIONAL VITAMIN D STATUS AND OFFSPRING BONE MINERAL MEASURES AT AGE 10-12 YEARS
Natalie Hyde¹, Sarah Hosking¹, Sharon Brennan-Olsen¹, John Wark² Kathy Bennett¹ A, Amelia Morse¹, Julie Pasco¹
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2 University of Melbourne

Developmental plasticity in utero is thought to play a role in future risk of osteoporosis. Thus, we aimed to determine the association between gestational vitamin D status and offspring bone mineral measures during childhood.

Data were collected from the Vitamin D in Pregnancy (VIP) study (2002-04); a cohort of 475 pregnant women, recruited from the Geelong Hospital in early pregnancy (gestation 12.6±2.8 weeks). Venous blood samples were taken at recruitment and 28-32 weeks. Maternal serum 25-hydroxyvitamin D (25(OH)D) was measured by radioimmunoassay (Immunodiagnostic Systems). Offspring (n=168) underwent an assessment of areal bone mineral density (BMD), aged 10-12 (11.0±0.46) years, by dual energy X-ray absorptiometry (GE Lunar).

A sex*25(OH)D interaction was observed in models predicting BMD thus data were stratified by sex. Correlations were observed for BMD at the lumbar spine and total body less head (TBLH) with 25(OH)D levels at recruitment in boys (r=0.21 p=0.05; r=0.22, p=0.05, respectively), but not girls (both p>0.4). After adjustment for offspring height, weight and age, 25(OH)D was positively associated with BMD at lumbar spine and TBLH in boys (β0.06±0.02, p=0.019; β0.05±0.02, p=0.005, respectively), but not girls (both p>0.05). All associations were independent of pubertal staging, birth weight, gestation length, season, maternal age, smoking and socioeconomic status at recruitment. 25(OH)D at 28-32 weeks showed no association with BMD.

Maternal vitamin D in early pregnancy displayed a sexually dimorphic effect; it was positively associated with bone accrual in male offspring but not females. This may predispose male offspring to an increased risk of osteoporosis in future.
The epidemiology of joint replacements in Western Victoria: the Ageing, Chronic Disease and Injury (ACDI) study

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2Australian Orthopaedic Association National Joint Replacement Registry, School of Population Health and Clinical Practice, University of Adelaide, Adelaide SA, Australia
3Data Management and Analysis Centre, University of Adelaide, Adelaide SA, Australia
4Faculty of Health, Deakin University, Melbourne VIC, Australia
5Royal North Shore Hospital, Sydney NSW, Australia
6Barwon Health, Ryrie Street, Geelong, VIC, Australia
7School of Nursing and Midwifery, Deakin University, Melbourne VIC, Australia
8Department of Orthopaedics, Barwon Health, Geelong VIC, Australia
9Southwest Orthopaedics, Warrnambool VIC, Australia

Background/Aim: In order for clinicians to effectively plan interventions, prevention strategies and policy initiatives to reduce gaps in service delivery, epidemiological data are needed. This is particularly crucial for rural areas, where accessibility of health services may be lower. The region of western Victoria includes both urban and rural communities, with varied lifestyle, health behaviours and settings. We have mapped the incidence of joint replacements across this region.

Methods: Data from the Australian Orthopaedic Association National Joint Replacement Registry (AOANJRR) were used to calculate age-standardised rates of joint replacements for men and women in western Victoria during 2011-13 inclusive. Incidence rates were age-standardised to the Australian 2011 Census Population and have been mapped, sub-divided into 21 Local Government Areas (LGAs) (Figure).

Results: Rates of joint replacements across LGAs were higher in women than men. In men, the highest joint replacements rates per 10,000 persons per year occurred in the LGAs of Glenelg (95.6), Horsham (95.7), Ararat (100.7) and Pyrenees (102.6). The lowest joint replacements rates in men occurred in Warrnambool (64.0). In women, the highest joint replacements rates occurred in Pyrenees (119.8) and Horsham (126.5). The lowest occurred in Hindmarsh (65.4). Accessibility/remoteness (ARIA) and socioeconomic scores (IRSAD) for LGAs were not predictive of joint replacements.

Conclusion: The patterns identified were not solely a reflection of accessibility/remoteness or differences in socioeconomic status. Further research is necessary to investigate if there is a mismatch between need and actual replacements, which will allow development of appropriate prevention and healthcare resource allocation.

Figure: The Ageing, Chronic Disease and Injury (ACDI) Study region. The region is divided into 21 Local Government Areas, listed at the right.
HUMAN STROMAL CELLS ISOLATED FROM SUBCHONDRAL BONE VARY BETWEEN PATIENTS IN MULTIPOTENCY AND THERAPEUTIC POTENTIAL TO AMELIORATE TENDON PATHOLOGY

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Introduction: Mesenchymal stem/stromal cells are touted, with little evidence, as effective therapies in a plethora of chronic diseases including tendinopathy. To progress to a rational therapy, we sought to isolate bone stromal cells and compare their functionality between individual patients.

Methods: Human stromal cell preparations (HSCs) were isolated from bone removed from patients (n= 13) undergoing knee or hip replacements. HSCs were expanded in culture and tested for colony formation and ability to differentiate into bone, fat and cartilage (histology and gene expression of differentiation markers). HSCs were also co-cultured with sheep tendon explants (n=5 per HSC) for 24 hours followed by real time RT-PCR for expression of tendon genes.

Results: HSCs exhibited diverse abilities to form colonies and to differentiate into fat, bone and cartilage. Chondrogenic (but not adipogenic or osteogenic) differentiation declined significantly with decreasing ability to form colonies (P=0.002). Effects of HSCs on stress-deprivation induced tendinopathic gene expression varied markedly between preparations. Stimulation of tendon COL2A1 expression positively correlated with the cells' chondrogenic ability (r=0.585; P=0.014). Increasing adipogenic capacity was associated with increased stimulation of tendon ADAMTS4 (r=0.518; P=0.040) expression, and decreased MMP13 (r=0.536; P=0.027) and COL2A1 expression (r=0.551; P=0.022).

Discussion: Stromal cells isolated from bone of patients undergoing joint replacement provided access to a clinically relevant autologous MSC source. Our results suggest that cells with increased chondrogenic capacity may enhance chondroid differentiation expression in tendon that would be detrimental. Greater adipogenic differentiation, on the other hand, may be an indicator of therapeutic potential.