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Bone Research Society
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Invited Abstracts

IS1

The EN1 Story: The gains from imputation and whole genome sequencing

Brent Richards

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Identifying genetic determinants of bone mineral density and fracture in humans will help to understand the etiology of this common and costly condition. The contribution of low-frequency and rare genetics variants to complex diseases like fracture have been hypothesized to have larger effects, yet due to technological hurdles little data was available to test this hypothesis.

Using what was the world's largest whole genome sequencing based study, we identified novel non-coding genetic variants with large effects on BMD (n=53,236) and fracture (n=508,253). We identified a novel locus in the human genome with low frequency non-coding variants near EN1. The effect sizes of these genetic variants were fourfold larger than the mean of previously reported common genetic variants and they also decreased the risk of osteoporotic fracture. We explored the causal role of EN1 by generating an En1cre/flox mouse model and observed that conditional loss of En1 results in low bone mass, probably as a consequence of high bone turnover.

In summary, EN1 is a major determinant of bone mineral density and fracture risk in humans. This talk will also present further evidence from genetic studies implicating EN1 in bone physiology.

IS3

Hypoxia signaling, blood vessels and bone

Geert Carmeliet

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Cell-based therapy is a promising strategy in regenerative medicine, but the poor survival of the implanted cells remains a major challenge and limits clinical translation. By increasing HIF signaling, we could enhance the survival and bone forming capacity of periosteal cells after implantation in a large bone defect. These properties relied on adaptations in glutamine and glucose metabolism to preserve redox and energy balance. Redox homeostasis is maintained by glutaminase-mediated glutathione synthesis whereas energy balance is preserved by increased glycogen storage. Targeting cellular metabolism is thus an appealing strategy for bone regeneration and cell-based therapy in general.

HIF signaling is also required for the survival of chondrocytes in the developing avascular growth plate. However, enhanced HIF signaling results in an energy deficit, reduced proliferation and altered matrix properties leading to shorter bones with increased bone mass. Bone growth and bone mass thus depends on well controlled HIF signaling.

IS5

Gut microbiota and bone metabolism

Claes Ohlsson, Klara Sjögren

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The gut microbiota (GM), the commensal bacteria living in our intestine, performs numerous useful functions, including modulating host metabolism and immune status. Our recent studies demonstrate that the GM is also a regulator of bone mass and we propose that the effect of the GM on bone mass is mediated via effects on the immune system, which in turn regulates osteoclastogenesis. A role of the GM in bone metabolism is further supported by studies demonstrating that antibiotic, probiotic, and prebiotic treatments that impact GM composition regulate bone metabolism. Collectively, these studies suggest that the GM may be a novel therapeutic target for osteoporosis. Treatment with probiotics has already been shown to improve bone mass in rodent models of bone loss, but future randomized clinical trials are required to determine the possible effect of probiotics and other novel therapies modulating the GM composition on bone mass and fracture risk in patients with osteoporosis.

Access to cheaper sequencing and improved bioinformatics tools will allow metagenomic sequencing for the analysis of the GM composition in large prospective clinical cohort studies. This can be used to evaluate the predictive value of the GM composition as a biomarker for low bone mass and fracture risk. In addition, metatranscriptomics and metaproteomics will most likely be used to identify the microbial genes and proteins that have an impact on bone mass and fracture risk.

We propose a new cross-disciplinary GM-bone research field called 'osteo-microbiology', bridging the gaps between bone physiology, gastroenterology, immunology, and microbiology. Future studies are clearly warranted in this new research field to determine if the GM composition might be used as a biomarker for fracture risk prediction and to validate the GM as a possible novel therapeutic target for osteoporosis.

IS7

Understanding cell senescence to identify potential new therapeutic strategies in bone ageing

Lynne Cox

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Cellular senescence is a programme of cell proliferation arrest that occurs in response to stressors including DNA damage, oncogene

Full programme book available at:
https://boneresearchsociety.org/download/brs-2017-full-programme/

induction, ER stress and oxidative stress, as well as telomere loss on replicative exhaustion. Senescent cells show altered patterns of gene expression and they develop a secretory phenotype (SASP) that includes production of a large number of pro-inflammatory cytokines and chemokines, as well as matrix remodeling factors. Senescence-associated loss of proliferative capacity of stem cells and progenitor cells, differentiation into adipocyte instead of osteoblast lineages and the pro-inflammatory SASP may all contribute to age-related osteoporosis and poor healing of fractures in the elderly. Consistent with this, removal of senescent cells from ageing mice improves their overall health including skeletal integrity, strongly supporting the idea that cell senescence directly contributes to ageing and age-related disease. Recently, senescent cells have been identified in ageing bone, while local removal of senescent cells from joints following ACL trauma has been shown to improve outcomes. To identify key factors in senescence pathways, we have conducted unbiased proteomics analyses of cells at various stages as they progress to senescence, and based on these data are developing novel strategies to reverse senescence, with possible application to ageing bone.

IS8

Running on time: the role of the circadian clocks in the musculoskeletal system

Qing-Jun Meng

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The night and day cycle governs the circadian (24 hourly) rhythm of rest/activity, physiology and metabolism in animals and humans. A central clock in the brain coordinates the rhythmic locomotion behaviour and synchronizes various local oscillators, such as those found in the musculoskeletal system. Disruptions to circadian rhythms (e.g. during ageing) have been linked to various diseases. Osteoarthritis and low back pain are among the most prevalent skeletal conditions associated with old age. However, the reasons why susceptibility to these diseases increases with age are not well understood. Work from our group has revealed a functional link between circadian clocks and the homeostasis of the articular cartilage and intervertebral disc tissue. We show that the daily rhythms in these tissues become dampened and out-of-phase during ageing. Moreover, mice with targeted knockout of an essential clock gene (Bmal1) in chondrocytes and disc cells have profound, yet tissue-specific degeneration. This new avenue of research holds potential to better understand the pathogenesis of these skeletal disorders.

IS9

Katherine Brooke-Wavell

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Exercise may benefit osteoporotic fracture risk by increasing bone strength and reducing fall risk. Many studies have examined the types and intensities of exercise needed to increase bone density- with the most effective being those that generate high strain rates in bone. The skeletal location of bone mineral, as well as its density, may affect bone strength and can be incorporated into strength estimates that have been related to fracture risk. Furthermore, localised loss at the femoral neck may predispose to fractures. Recent research using hip structural analysis from dual X-ray absorptiometry scans and quantitative computed tomography suggests that exercise also produces localised adaptation that may affect bone strength independently of bone mineral density. Exercise may thus have the potential to target bone gains to specific skeletal sites where bone loss can predispose to osteoporotic fracture. Further research is needed to determine the exercise parameters that may stimulate bone accrual at sites of particular structural importance.

IS11

Bone marrow adipose tissue: starving for attention?

William Cawthorn

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Bone marrow adipose tissue (MAT) accounts for up to 70% of bone marrow volume and over 10% of total adipose mass in lean, healthy humans. MAT further increases in diverse clinical conditions, including ageing, osteoporosis, glucocorticoid treatment, cancer therapy and, strikingly, during caloric restriction. Many of these conditions are also associated with bone loss and increased fracture risk, and therefore it has been suggested that MAT might directly impact skeletal remodelling. Recent studies also support a role for bone marrow adipocytes in modulating haematopoiesis, fracture repair and progression of skeletal metastases or myeloid tumours. However, study of MAT has been relatively limited, and therefore the formation and function of bone marrow adipocytes remains poorly understood.

We previously revealed that, during caloric restriction, MAT contributes to increased circulating levels of adiponectin, a hormone with diverse cardio-metabolic and anti-inflammatory effects. Thus, like white adipose tissue, MAT is an endocrine organ that can exert systemic effects. My lab is now building on this finding by further investigating the causes and consequences of MAT formation, in particular during caloric restriction, with the goal of determining how MAT impacts metabolic and skeletal health. By combining preclinical models and clinical sample analyses, our research is beginning to reveal new insights into the putative endocrine functions of MAT; the mechanisms contributing to MAT formation; and the relationship between MAT accumulation, bone loss and metabolic health.

Submitted abstracts

Oral presentations

OC1

Genome-wide association analysis identifies *CXCR4* gene as predictor of therapeutic response to teriparatide in severe osteoporosis

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Teriparatide (TPTD) is an anabolic agent associated with increased bone formation and reduced fracture rates in comparison to oral bisphosphonates. However, treatment costs are high and the

response is variable. We aim to identify markers of response to TPTD to target treatment more effectively. A genome-wide association study was performed in 442 patients with osteoporosis from UK, Denmark and Slovenia, using an Illumina OmniExpress Exome v8 array. The primary outcome was change in spine BMD (LS-BMD). This was assessed after application of standard quality control measures using a linear regression association analysis in PLINK using residuals of percentage of change in spine BMD per month, standardised by centre, principal components, and age. We identified a SNP on chromosome 2 showing significant association at genome-wide level with response to TPTD therapy in the discovery dataset ($p=9.28x10^{-10}$, beta=-0.38, 95%CI= [-0.51--0.26]). No evidence of genomic inflation was observed ($\lambda=1.09$). Three additional suggestive signals were identified on chromosomes 8, 13 and 15, with p-values<$5x10^{-6}$. Combined information from the top four signals showed a highly significant association of change in LS-BMD with the number of alleles carried (1.5% increase in LS-BMD at 24-month in individuals carrying 5 non-responder alleles compared with 17.5% increase in LS-BMD in those carrying one or none non-responder alleles ($p=2.2x10^{-16}$)). The top hit was found to be an e-QTL for *CXCR4* expression in peripheral blood cells (Z-score=-3.15, p=0.0016). *CXCR4* encodes for a chemokine receptor specific for stromal cell-derived factor 1 expressed in osteoblasts and previously implicated in the activation of WNT signalling and bone formation. Replication studies are now in progress involving 220 TPTD treated patients to confirm and extend these findings. At present we have identified a genome wide significant predictor of response to TPTD and a promising allelic score that identifies patients with a 10-fold difference in treatment response. If confirmed by replication studies that are currently in progress, genotyping for these risk alleles could be of clinical value in personalising treatment options in patients being considered for TPTD therapy.

OC2

RXRA promoter DNA methylation at birth is associated with gestational vitamin D supplementation: results from the MAVIDOS trial

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Objectives: We tested whether maternal supplementation with vitamin D during pregnancy would lead to altered perinatal DNA methylation at the retinoid-X-receptor-alpha (*RXRA*) gene, which has a key role in the nuclear action of $1,25(OH)_2$-vitamin D.

Methods: The Maternal Vitamin D Osteoporosis Study (MAVIDOS) is a randomised, double-blind, placebo-controlled trial of 1000iu/day cholecalciferol vs matched placebo from 14 weeks gestation until delivery. Umbilical cord tissue from the fetal side was collected at birth and frozen at -80°C (n=436). Pyrosequencing was used to measure DNA methylation at 10 CpG sites within the RXRA promoter. CpGs sites were selected on basis of those previously analysed in the observational Southampton Women's Survey mother-offspring cohort, in which associations between RXRA methylation and offspring bone development were seen. Independent t-tests were used to assess the differences in methylation between the treatment groups.

Results: Statistically significant ($p\leq0.05$) differences in methylation at the RXRA region of interest were observed between the cholecalciferol and placebo group at 5 of 10 CpG sites. Overall, methylation levels were significantly lower in the umbilical cord from offspring of cholecalciferol supplemented mothers: e.g. at RXRA CpG -2642, mean difference in methylation between the supplemented and placebo groups was -2.1% (n=433, 95% CI -3.7 to -0.3, p=0.02). We have previously shown, using electrophoretic mobility shift assays, that methylation in this region leads to reduced transcription factor binding. Therefore, the reduced methylation observed in the supplementation group may be associated with an upregulation of $1,25(OH)_2$-vitamin D signalling.

Conclusions: Our findings support previous observational results and provide new evidence that maternal gestational supplementation with cholecalciferol leads to altered perinatal epigenetic marking. These results inform potential mechanistic pathways linking maternal 25(OH)D to offspring bone mass, and may yield novel biomarkers of future bone development.

OC3

The nuclear receptor PPARα is a regulator of bone volume and strength

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Bone quantity and quality is tightly linked to the metabolic activity and signalling of cells within the bone marrow microenvironment. The Peroxisome Proliferator-Activated Receptor family (PPAR-α, -β, -γ) are nuclear transcription factors directly activated by specific biolipids and targeted clinically to treat metabolic disorders such as diabetes. While PPARβ and PPARγ have recently been shown to regulate bone cell differentiation and homeostasis, the role of PPARα in bone biology is poorly characterised. Moreover, PPARα is the direct target of the fibrate class of drugs, including the widely prescribed cholesterol-lowering fenofibrate (FF), yet the mechanism governing purported skeletal effects of FF remain unclear. We investigated the hypothesis that PPARα plays a regulatory role in bone using in vitro cellular and molecular studies combined with micro-CT scanning, biomechanical testing and histomorphometric analysis of 3 wk, 12 wk and 60 wk male and female PPARα-deficient mice ($PPAR\alpha^{-/-}$). At 3 weeks of age $PPAR\alpha^{-/-}$ mice display reduced cortical thickness ($PPAR\alpha^{+/+}$:5.87±0.06μm, $PPAR\alpha^{-/-}$:5.18±0.12 μm, p<0.001) and trabecular bone BV/TV ($PPAR\alpha^{+/+}$: 8.35±0.30%, $PPAR\alpha^{-/-}$:6.22±0.27%, p<0.001). PPARα humeri were not as strong as wildtype (Maximum load: $PPAR\alpha^{+/+}$ 5.25±0.12N; $PPAR\alpha^{-/-}$ 3.80±0.09N; p<0.001), while bone stiffness was significantly decreased (Modulus of Elasticity: $PPAR\alpha^{+/+}$ 3426±102.6MPa;

PPARα$^{-/-}$ 2299±89.32MPa; p<0.001). At 12-weeks and 60-weeks of age cortical density and bone strength remained significantly reduced, after taking into account sex and weight related confounding. Histomorphometric analysis revealed 45.7% fewer osteoblasts and a 76.5% increase in osteoclasts (p<0.05) in aged PPARα$^{-/-}$ long bones compared to wildtype, with no significant change in adipocyte number. In addition, FF significantly increased 2T3 mouse preosteoblast mineralisation in a dose-dependent manner from 10 to 25uM (p<0.001), without altering cell proliferation. In comparison, ATDC5 chondrocyte proteoglycan production was reduced by FF treatment. Our results suggest that PPARα regulates bone development and remodelling throughout life, and that FF may improve bone quality with ageing and protect against diabetes-induced bone loss, by activating PPARα.

OC4

Does skeletal NPP1 regulate mineralisation and energy metabolism?

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We recently demonstrated that Ectonucleotide pyrophosphatase/phosphodiesterase-1 (NPP1)-deficient mice display long bone hypomineralisation, soft tissue calcification and insulin sensitivity. We therefore hypothesise that osteoblast-derived NPP1 suppression inhibits bone mineralisation whilst counteracting insulin-resistance.

Wild type murine calvarial osteoblasts were cultured for 28d (2.5mM βGP;50 μg/ml ascorbic acid). Gene expression was determined by qPCR. Calcium deposition was measured by quantitative alizarin-red staining, and alkaline phosphatase (ALP) activity was determined. Mice lacking osteoblast-specific NPP1 were generated by crossing *Enpp1*$^{flox/flox}$ and *Osteocalcin*-Cre mice (*Ocn-Cre;Enpp1*$^{flox/flox}$). Metabolic tests, fat-pad analyses, histopathology, gait analysis, micro-computed tomography and mechanical testing of bone were undertaken in male and female *Ocn-Cre;Enpp1*$^{flox/flox}$ and compared to *Enpp1*$^{flox/flox}$ mice up to 22-weeks of age.

In vitro osteoblast studies demonstrated elevated calcium deposition, ALP activity and osteoblast marker mRNA expression (*Ocn, Runx2, Coll1a*) over 7d (p<0.05). Concomitantly, *Enpp1* expression was increased (5.6 fold, p<0.01). 22-week-old female *Ocn-Cre;Enpp1*$^{flox/flox}$ femurs showed increased bone volume (101%, p<0.001), trabecular number (82%, p<0.001) and trabecular thickness (11%, p<0.001), with decreases in structural model index (19%, p<0.001). *Ocn-Cre;Enpp1*$^{flox/flox}$ cortical bone thickness (12%, p<0.05) and periosteal/endosteal diameters were also increased (p<0.001). Work to rupture was also increased in *Ocn-Cre;Enpp1*$^{flox/flox}$ (p<0.05). Epiphyseal trabecular number (11%, p<0.01), medial and lateral subchondral bone volume (30%, p<0.05) and thickness (25%, p<0.01) were all increased, consistent with osteoarthritis. Gait analysis revealed increased base of support (15%, p<0.05), girdle support (66%, p<0.05), initial (117%, p<0.05) and terminal (128%, p<0.05) forelimb dual stance suggesting stance instability. Subcutaneous, gonadal and mesenteric fat pad weights were reduced in 6-week old *Ocn-Cre;Enpp1*$^{flox/flox}$ mice (p<0.05), however no notable difference in insulin or glucose sensitivity was observed. No histopathological abnormalities were observed in spine, kidney, aorta and patella.

Our data suggests a functional role for osteoblast-derived NPP1 as a local inhibitor of bone mineralisation, an action overshadowed by the pathological phenotype of the global *Enpp1*$^{-/-}$ mouse. Osteoblast-specific ablation of NPP1 may also increase susceptibility to osteoarthritis, regulate fat accumulation and gait stability. However, the soft tissue calcification and improved insulin sensitivity phenotypes associated with global NPP1 deficiency are likely due to the actions of non-skeletal NPP1.

OC5

The proton pump inhibitor omeprazole inhibits PHOSPHO1 activity and matrix mineralisation *in vitro*

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Objectives: Recent epidemiological evidence has revealed that gastric acid-suppressive proton pump inhibitors (PPIs) are associated with an increased risk of osteoporosis-related bone fractures. Current dogma implicates an impairment of calcium absorption through suppression of gastric acid production, however here we investigated whether the bone-specific phosphatase PHOSPHO1 mediates the bone response to PPI administration.

Methods: The effects of the commonly prescribed PPI omeprazole on PHOSPHO1 and TNAP activity were assessed by phosphatase assays. Concurrent experiments were conducted with the histamine-2 receptor antagonist (H2RA) ranitidine. Primary calvaria osteoblasts were treated with increasing concentrations (0-10 μM) of omeprazole and mineralisation was assessed by Alizarin Red staining and subsequent quantification.

Results: The PPI omeprazole was found to be a potent inhibitor of PHOSPHO1 activity with an IC50 of 2.803 μM. Omeprazole did not however inhibit TNAP activity (at concentrations up to 10μM), therefore suggesting that any potential effects on mineralisation are PHOSPHO1 dependent. The H2RA ranitidine acid which has not been associated with an increased risk of fracture had no effect on PHOSPHO1 or on TNAP activity, at concentrations up to 10 μM. Assessment of matrix mineralisation in primary osteoblast cultures was consistent with the phosphatase activity assays: whilst control-treated cultures formed mineralised nodules after 28 days in culture, the addition of 10 μM omeprazole significantly inhibited matrix mineralisation (P<0.05). Nodules were clearly visible throughout these cultures suggestive that the effects seen are directly on mineralisation rather than the differentiation of the cells.

Conclusion: This study has revealed for the first time that the PPI omeprazole inhibits the activity of PHOSPHO1 and bone mineralisation *in vitro* whereas the H2RA ranitidine had no significant effect on PHOSPHO1 at concentrations of up to 10 μM. This raises the possibility that PPI induced inhibition of PHOSPHO1 might contribute to the increases risk of fracture in patients being treated with PPI. Current studies are in progress to establish the *in vivo* effects of PPI administration on PHOSPHO1 and matrix mineralisation.

OC6

The adenosine A2B receptor (ADORA2B) drives osteoclast-mediated bone resorption in hypoxia

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Objectives: Osteoclast-mediated bone resorption is enhanced in the hypoxic microenvironment of diseases including rheumatoid arthritis and bone metastatic cancer. This increased resorption is driven by the hypoxia-inducible transcription factor, HIF. ADORA2b is a HIF-responsive gene that is upregulated in hypoxic osteoclasts. ADORA2b is only functional in hypoxia or inflammatory conditions, as only then are activating extracellular concentrations of adenosine achieved by hydrolysis of extracellular ATP. Given the increasing interest in musculoskeletal effects of extracellular ATP, we investigated whether ADORA2b plays a role in osteoclast-mediated resorption of bone.

Methods: Human osteoclasts were differentiated from CD14+ monocytes using M-CSF and RANKL. Hypoxic induction of ADORA2b was determined by microarray, real-time PCR and Western blot, with isoform-specific HIF-1α and HIF-2α siRNA establishing HIF-dependence. Effects of specific ADORA2b inhibitors (MRS1754, PSB603) were assessed on osteoclast formation (number of TRAP- or VNR-positive multi-nucleated cells) and bone resorption (toluidine blue staining of dentine discs). Reciprocal regulation of HIF-mediated transcriptional activity was assessed by luciferase assay.

Results: Microarray experiments identified 2.7-fold ($p<0.002$) induction of ADORA2b (NM_000676.2) in hypoxic osteoclasts (2% O_2, 24 h). This was confirmed by real-time PCR (3.8-fold±1.9, $p<0.01$) and Western blot and prevented by transfection with HIF-1α ($p<0.001$) or HIF-2α ($p<0.01$) siRNA. Hypoxia increased ATP secretion from CD14+ monocytes (240 min, $p<0.05$) and osteoclasts (60 min $p<0.01$) and increased expression of CD39 ($p<0.05$) and CD73 ($p<0.01$) mRNAs, ectonucleotidases responsible for converting extracellular ATP into adenosine. ADORA2b inhibition prevented the hypoxic increase in osteoclast-mediated bone resorption (MRS1754, $p<0.001$), partly by reducing hypoxic glycolysis via inhibition of HIF transcriptional activity. ADORA2b inhibition also reduced osteoclastogenesis in hypoxia by inhibiting cell fusion (MRS1754, day 3-4, $p<0.001$). Under normoxic conditions ADORA2b inhibition did not affect any parameter tested.

Conclusion: ADORA2b inhibition restrains both osteoclastogenesis and bone resorption within the hypoxic microenvironment. As ADORA2b is only active in hypoxic and/or inflammatory conditions, ADORA2b inhibition could specifically prevent the pathological osteolysis associated with hypoxic diseases including rheumatoid arthritis and bone metastatic cancer.

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OC7

Osteoclast ruffled border formation: a new perspective

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The osteoclast ruffled border (RB) is a highly folded, dynamic membrane, circumscribed by an actin ring, which forms as osteoclasts undertake bouts of resorptive activity while moving over the bone surface. At the RB, osteolytic enzymes are released and degraded bone is endocytosed. We investigated how osteoclasts form and fold membrane into a RB using TEM (transmission electron microscopy), electron tomography (ET) and live cell imaging. Mature osteoclasts were isolated from rabbit bone and cultured on dentine discs. Osteoclasts were also generated from bone marrow of transgenic mice expressing GFP-labelled LC3, a membrane protein localised to the autophagosomal membrane. Mature rabbit osteoclasts were treated with calcitonin to abolish the RB followed by calcitonin washout which allowed for RB reformation to be synchronised. TEM and ET showed that immediately after calcitonin treatment and washout the RB had disappeared and the cytoplasm had become highly vacuolised. At 80 minutes post-washout, vacuolisation decreased and fusion of vacuoles and vesicles containing electron dense material resulted in the formation of an intracellular membrane complex near the bone surface. By 120 minutes, channels connected this complex with the bone-facing plasma membrane with some channels containing electron dense material. Thereafter, more channels appeared eventually forming the distinct membrane folds of a mature RB. Live cell imaging of LC3-GFP osteoclasts showed that LC3 localised briefly within developing actin rings and then disappeared. TEM examination showed that LC3 localisation could not be explained by autophagosome fusion indicating that LC3 may target directly to the forming RB in an autophagy-independent manner. Taken together, our data obtained using these three advanced imaging techniques suggest that prior to initiation of resorption, osteoclasts form an intracellular membrane complex which acts as a docking station for vesicles containing bone degrading products. Formation of channels allows release of these products onto the bone surface and leads to the membrane folds that are characteristic of a RB. The newly identified time-dependent localisation of LC3 in the early stages of this process indicates LC3 may be facilitating membrane folding and/or vesicular fusion and requires further investigation.

OC8

AdipoR1 deficiency leads to low bone mass and increased bone adiposity *in vivo*

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The fat-derived adipokine adiponectin plays a protective role in several disease states, with circulating levels correlating negatively with diabetes and obesity and positively with longevity. Many of these effects are known to occur via adiponectin receptor 1 (AdipoR1), the most ubiquitous and highly expressed adiponectin receptor in the body, which in turn mediates its downstream effects, such as insulin sensitisation, via AMPK activation.

The effects of adiponectin in bone are still unclear with several *in vivo* models, including adiponectin knockout mice, and *in vitro* techniques reporting conflicting results. To investigate the true effects of adiponectin in the musculoskeletal system more clearly, we used genetically modified AdipoR1$^{-/-}$ and control female C57Bl6 mice at young (1 month) and adult (6 month) ages. Long bones were analysed by histomorphometry, micro-CT scanning and biomechanical (three-point bend) testing to investigate our hypothesis that loss of AdipoR1 leads to abnormal bone formation and maintenance.

Despite AdipoR1$^{-/-}$ mice showing no developmental differences to wild-type controls, AdipoR1$^{-/-}$ tibiae demonstrated a significant

decrease in trabecular BV/TV as compared to controls at both 1 (35%, p<0.01) and 6 months (73%, p<0.01) with significant alterations in trabecular number (0.0146±0.0022/mm vs. 0.0083±0.0006/mm), thickness (36.0±1.7 mm vs. 23.0±2.6 mm) and spacing (43.5±3.5 mm vs. 62.9±1.3 mm). A reduction in cortical thickness at the mid-diaphysis was also observed upon AdipoR1 deletion (237.7±8.0 mm vs. 200.0±6.4 mm, p<0.01), and supported by biomechanical analysis which revealed a significant decrease in bone strength (16.5±0.4N vs. 13.9±0.6N maximum load, p<0.01) and stiffness (13129±209MPa vs. 12313±319.5MPa, p<0.05) in AdipoR1$^{-/-}$ mice versus controls. Interestingly, histomorphometric analysis revealed a shift in cellular distribution with osteoclast parameters remaining unchanged whilst adipocyte number and size were increased (114% and 127% increase respectively, p<0.05) and osteoblast number (42%) and surface area (33%) were decreased in AdipoR1$^{-/-}$ mice compared to control.

Our study suggests that loss of AdipoR1 leads to impaired bone formation due to increased adipocyte and decreased osteoblast formation, implying an alteration in mesenchymal stem cell lineage commitment and differentiation.

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OC9

Sarcopenia is negatively related to osteogenic impacts achieved through habitual physical activity: findings from a population-based cohort of older females

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Objectives: We aimed to determine if sarcopenia, or its components, predict exposure to higher vertical impacts achieved through habitual physical activity (PA), following our recent finding that higher impacts are positively related to bone strength in older women.

Methods: Participants were older women from the Cohort of Skeletal Health in Bristol and Avon. The European Working Group on Sarcopenia in Older People criteria was used to define sarcopenia (appendicular lean mass index (ALMI) ≤5.45 kg/m^2, measured by DXA, plus gait speed <0.8m/s and/or grip strength <20 kg). In a subset, lower limb muscle function was assessed by jumping mechanography (JM). PA was assessed by tri-axial accelerometry and higher impact activity defined as the number of vertical accelerations ≥1.5 g normalised to one week. Associations between sarcopenia/muscle function and PA were analysed by multivariable linear regression adjusted for age, height and weight (or fat mass for models including ALMI), comorbidities, smoking, alcohol and Index of Multiple Deprivation.

Results: 380 women, mean age 76.7 (SD3.0), had complete data for sarcopenia status and higher impact counts, of whom 242 also completed JM assessment. Sarcopenia was associated with reduced higher impact counts [β^a=0.39(95% CI 0.21, 0.70), p<0.01] (β^a=ratio of geometric means). In terms of individual components of sarcopenia underlying this relationship, gait speed [β^b=1.56(1.26, 1.94), p<0.01] and grip strength [β^b=1.22(1.01, 1.49), p=0.04] were positively related to higher impacts (β^b-1=fold increase in impact counts per SD increase in exposure). In addition, JM-assessed peak force was positively related to high impacts [β^b=1.50(1.15, 1.95), p<0.01], whereas no association was seen for peak power [β^b=1.22(0.93, 1.59), p=0.15]. Multivariate analyses identified gait speed [β^b=1.47(1.14, 1.89), p<0.01] and peak force [β^b=1.40(1.07, 1.84), p=0.02] as independent predictors of higher impacts.

Conclusions: Older women with sarcopenia experience fewer bone-strengthening impacts during habitual PA, reflecting a combination of their lower gait speed and reduced lower limb peak force. These findings suggest that, to improve bone strength in this group, PA interventions need to incorporate strategies to both increase walking speed and lower limb muscle strength.

OC10

The longevity-related SirT1 enzyme retards inflamm-ageing *in vivo*

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Ageing is accompanied by an increase in inflammatory status. This underlying chronic inflammation may fuel age-related disorders e.g. diabetes, heart disease and arthritis. However, the cause of this inflamm-ageing is unknown. We hypothesized that factors linked to ageing and lifespan may also control the normal inflammatory response, where dysregulation with ageing may contribute to an elevated inflammatory state. The class III deacetylase SirT1 is strongly linked to longevity and can extend lifespan in various model systems when over-expressed or stimulated pharmacologically. Our work has shown that SirT1 expression decreases within the ageing skeleton with increasing age and dysregulates bone remodelling to predispose to age-related bone loss. In addition, loss of SirT1 increases the activity of the common inflammatory mediator NFkB, in vivo.

Using a novel, inducible whole-body SirT1deletion mouse model (SirT1$^{fl/fl}$ x ROSA CreER^{T2}), we examined the inflammatory response within the joint by histomorphometric analysis and profiled inflammatory gene signatures using a low density qPCR array.

SirT1 deficiency increased fibrosis (p<0.05), joint stiffness (p<0.05) and synovitis (p<0.001) score and several inflammatory response genes including IL-1β (p<0.0001), IL-6 (p<0.0001), TNF-α (p<0.0001), ADAMTS-4 (p<0.001) and MMP-13 (p<0.01). To assess the accumulative contribution of mechanical joint injury, SirT1-deficient and WT mice underwent joint destabilisation surgery (DMM), an established model of human osteoarthritis (OA). Surprisingly, SirT1-deficient mice) were protected from disease 12 weeks post-DMM compared to control mice, as evidenced by reduced OARSI disease scoring (4.462±0.8291 Vs 15.58±1.411; p<0.0001). Furthermore, bone marrow chimera experiments showed SirT1-deficient mice receiving WT bone marrow displayed no increase in the early synovitis and inflammatory gene expression observed in non-transplanted mice, but did show increased long term disease scores (p<0.05), and where the protective nature of SirT1 deficiency post-DMM was lost. The opposite effect was observed in WT mice engrafted with SirT1-deficient bone marrow (p<0.05).

These results suggest that SirT1 has anti-inflammatory effects under normal conditions, and that loss of SirT1 with increasing lifespan leads to an inflamm-ageing phenotype. Interestingly, the increased fibrosis and stiffness of the joint following SirT1 deletion served to stabilise and protect against experimental OA.

OC11

Relationships between markers of inflammation, grip strength and bone microarchitecture: findings from the Hertfordshire Cohort Study

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Objectives: To examine the association between indices of inflammation, grip strength and bone mineral density (BMD) in a population-based cohort of older adults in the United Kingdom.

Methods: Participants were recruited from the Hertfordshire Cohort Study. Dual energy X-ray absorptiometry (DXA) was performed at the lumbar spine and proximal femur for 365 participants at baseline and repeated at a median interval of 4.5 years (IQR 3.6 to 5.2). DXA outcomes included both level and change in both total lumbar spine and total femoral neck BMD.

Grip strength was measured in 335 participants at baseline and follow-up (median follow-up time: 10.8 years [IQR 10.2 to 11.6]) and change in grip strength was ascertained using a residual change approach.

Inflammatory markers were ascertained at baseline using enzyme-linked immunosorbent assay (ELISA) techniques and Bio-Plex Pro Assays. Gender-adjusted linear regression was used to examine the associations between inflammatory markers and outcomes with and without adjustment for anthropometric and lifestyle factors.

Results: The mean (SD) ages at baseline were 64.4 (2.5) and 66.5 (2.7) years for men and women in the DXA group, and 63.8 (2.5) and 65.6 (2.7) years in the grip strength group, for men and women respectively. Higher levels of CRP were associated ($p<0.04$) with lower grip strength at follow-up and accelerated decline in grip strength from baseline to follow-up.

Higher levels of IL-1β and adiponectin:leptin ratios were each associated with lower baseline lumbar spine and femoral neck BMD in gender-adjusted ($p<0.04$) and fully-adjusted ($p<0.05$) analyses. Higher levels of IL-8 and lower levels of TNF were each associated with accelerated decline in lumbar spine BMD in both gender-adjusted ($p<0.02$) and fully-adjusted ($p<0.05$) analyses.

Conclusion: In a cohort of older adults, raised pro-inflammatory mediators (IL-1β and IL-8) and adiponectin:leptin ratio were associated with lower baseline BMD, and accelerated decline in BMD at the lumbar spine. Additionally, higher CRP was associated with poorer grip strength and accelerated decline. This adds weight to the theory that bone and muscle health can be influenced by both immune activation and alterations in adipokine homeostasis.

OC12

HMGB1 accelerates regeneration of multiple tissues by transitioning stem cells to G(Alert)

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Objectives: Our objective was to identify the factor that transitions endogenous quiescent G(0) stem cells to an "alert" phase thereby enhancing regeneration in multiple tissues, including bone, muscle and blood.

Methods: Alarmins are endogenous molecules released upon tissue damage. We screened candidate alarmins in-vitro and assessed the effects of local addition of the alarmin HMGB1 in murine models of skeletal, muscle and haematological injury. To further interrogate the role of HMGB1, we generated conditional HMGB1$^{-/-}$ to evaluate the effects of its absence in skeletal injury. The ability of HMGB1 to transition murine and human stem cells to G(Alert) was assessed using BrdU incorporation, FACS, measurement of cell size, ATP levels, mitochondrial DNA, and mTORC1 dependency. The receptor through which HMGB1 promoted healing was identified using small molecule inhibitors.

Results: HMGB1 and S100A8/9 levels were elevated post fracture in both human and murine samples. Only HMGB1 pre-treatment improved the osteogenic differentiation of human mesenchymal stem cells (hMSCs) in-vitro and local administration of HMGB1 accelerated fracture healing in-vivo via the CXCL12-CXCR4 axis. We confirmed our finding using conditional HMGB1$^{-/-}$ mice. Analyses of cell cycle kinetics, cell size, ATP levels, mitochondrial DNA, and mTORC1 dependency showed this was due to HMGB1 transitioning the murine skeletal stem cell to G(Alert). This effect also extended to other stem cell compartments, including murine muscle and haematopoietic stem cells, and human haematopoietic stem and progenitor cells, and hMSCs. HMGB1 also accelerated recovery in murine models when administered at the time, or 2 weeks before skeletal, muscle or haematological injury.

Conclusion: We have shown that injury leads to the release of HMGB1 and the administration of this alarmin accelerates healing in models of skeletal, muscle and haematological injury, even if administered 2 weeks before injury. HMGB1 leads to improved healing via the CXCL12-CXCR4 axis by transitioning a variety of stem cells to G(Alert). We confirmed our findings using small molecule inhibitors and conditional HMGB1$^{-/-}$ mice. Our results identified HMGB1 as a crucial factor for 'alerting' stem cells and highlight its therapeutic potential to accelerate the regeneration of multiple tissues following trauma, chemotherapy or elective surgery.

OC13

Effect of early bisphosphonate treatment on fracture healing: The fracture and bisphosphonate (FAB) study

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Background: There is currently no consensus on whether bisphosphonate therapy should be withheld following a fracture due to the possibility that it may impair fracture healing. Here we conducted a randomised placebo controlled trial to determine if treatment with the bisphosphonate alendronic acid affected healing in patients with wrist fracture.

Methods: The fracture and bisphosphonate (FAB) trial involved 421 patients aged ≥50 years of age with a distal radius fracture recruited from 15 centres in the UK. Consenting participants were randomised to receive alendronic acid 70mg weekly (n=215) or placebo (n=206) within 14 days of the fracture and were reviewed at 2, 4, 6, 8 and 24 weeks. The primary outcome was fracture healing on x-ray at 4-weeks assessed by observers blinded to treatment allocation. Secondary outcomes included the Disability Arm Shoulder and Hand (DASH) score, range of wrist movement, grip strength, pain, the prevalence of chronic regional pain syndrome (CRPS) and malunion. The trial was powered to detect a difference of 15% in rate of fracture healing between the groups since we felt this would be clinically significant.

Results: The baseline demographics and fracture characteristics of the two groups were similar. At 4 weeks 389 patients (92%) were available for analysis compared with 380 (90%) at 24 weeks. Adherence to study treatment was 86.9% (n=366). There was no statistically significant difference in the proportion of patients with fracture healing at 4 weeks between the groups: Alendronic acid, mean [95% CI]=23.8% [17.9 to 29.6%] vs. placebo=27.8% [21.4 to 34.2%] (p=0.53). The difference between groups was -4.1% [-12.8 to +4.7%]. There was similarly no significant difference in fracture healing at other time points or in DASH score, pain, grip strength, malunion rates or the prevalence of CRPS

Conclusions: We conclude that early administration of alendronic acid following wrist fracture does not have a clinically important effect on fracture healing. Our data suggest that bisphosphonates can be given almost immediately after fracture without compromising healing with potential benefits in the prevention of further fractures.

LB1

Characteristion of Angiopoietin-like 4 (ANGPTL4) as a potential new therapeutic target in osteosarcoma

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Objectives: ANGPTL4 is pro-tumourigenic in many primary tumours and is regulated by hypoxia-inducible factor-1 alpha (HIF-1α). As hypoxia and over-expression of HIF-1α are features of osteosarcoma, we hypothesize that ANGPTL4 might be highly expressed in osteosarcoma, associate with disease progression and might be a new therapeutic target in osteosarcoma. We have therefore studied ANGPTL4 expression in osteosarcoma. We have investigated it effects on the proliferation and migration of osteosarcoma cell lines, as well as its effects on their osteoblastogenic differentiation and stimulation of osteoclastogenesis.

Methods: ANGPTL4 expression in osteosarcoma tissue microarrays was determined by immunohistochemistry. Effects of hypoxia on ANGPTL4 secretion by osteosarcoma cell lines (MG63, MHM, HOS, OS18, ZK58, OSA and HOS-143B) was tested by ELISA. Regulation of ANGPTL4 by HIF was investigated using HIF inducers and isoform specific HIF siRNA (HIF-1α, HIF-2α). Effects of ANGPTL4 on cell proliferation, migration and stimulation of osteoblastogenesis was assessed in cells stably transfected with ANGPTL4 shRNA or an ANGPTL4 expression plasmid or treated with exogenous ANGPTL4. Osteoclastogenic differentiation of monocytes was assessed by TRAP staining.

Results: ANGPTL4 was expressed in 84/109 osteosarcoma cases. ANGPTL4 secretion by osteosarcoma cell lines increased in a time and oxygen severity-dependent manner and was the highest in MG63. ANGPTL4 was regulated by both HIF-1α and HIF-2α in MG63 (ANGPTL4 secretion was 17.32±7.13 ng ANGPTL4/mg protein [HIF-1a siRNA], 13.44±0.49 ng/μg [HIF-2α siRNA] and 54.19±22.54 ng/μg[control], p<0.05). ANGPTL4 over-expression increased the growth rate (1.86 fold increase, p<0.05) and migration (1.39 fold increase, p<0.05) of MG63. Both exogenous nANGPTL4 (100 ng/ml) and the conditioned medium of MG63 transfected with an ANGPTL4 expression plasmid enhanced monocyte proliferation (p<0.05). nANGPTL4 also enhanced osteoblastogenic differentiation of OSA cells and 200 ng/ml nANGPTL4 enhanced OC differentiation.

Conclusion: ANGPTL4 is over-expressed in osteosarcoma and stimulates proliferation and migration of osteosarcoma cells as a target gene of HIF. It also promotes osteolysis and therefore could be considered a novel therapeutic target in osteosarcoma.

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Clinical case studies

CC1

Hungry bone syndrome post-parathyroidectomy in a patient resistant to bisphosphonate therapy

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Background: Intravenous bisphosphonate given pre-operatively is thought to help to prevent the development of hungry bone syndrome (HBS) following parathyroidectomy. This a case of an 83 year old man who underwent a parathyroidectomy, having been resistant to bisphosphonate therapy and intolerant of cinacalcet, and developed prolonged HBS requiring intravenous and oral calcium supplementation, resulting in an eight week hospital admission.

Presenting problem: The patient had had primary hyperparathyroidism, osteomalacia and osteoporosis since at least 1976. He underwent a partial thyroidectomy (1999) to remove two parathyroid adenomas, but his PTH and ionised calcium (iCa) remained persistently elevated (26.7 pmol/L, 1.35 mmol/L, respectively). He remained asymptomatic, therefore was managed conservatively.

In 2011 his PTH and corrected calcium (CCa) had increased (61 pmol/L, 2.65 mmol/L respectively). Alendronate was commenced, his CCa remain unchanged (2.66 mmol/L). From 2012-2014 he tried cinacalcet but despite it normalising his CCa he was unable to tolerate it. A zoledronate infusion was given, CCa remained elevated (3.08 mmol/L). By October 2015 he had significant polyuria and polydipsia (CCa 3.25 mmol/L). A further zoledronate infusion was given but the CCa remained elevated. Imaging showed two large parathyroid adenomas bilaterally. He underwent a parathyroidectomy. Pre-operatively (CCa 3.26 mmol/L) he was given intravenous fluids, furosemide and calcitonin. Day 1 post-operative CCa 1.61 mmol/L, despite oral calcium and alfacalcidol. He continued on these and calcium gluconate infusions. During his admission his CCa fluctuated (1.29-1.84 mmol/L), warranting ongoing oral and intravenous supplementation. The CCa began to stabilise eight weeks post-operatively, at which point he was discharged on oral calcium supplementation. Four weeks after discharge his CCa was within the reference range.

Discussion: Intravenous bisphosphonates administered prior to parathyroidectomy are thought to possibly prevent post-operative HBS. There are case studies which support this, others do not. There are no case studies where the patient has apparent bisphosphonate resistance, which raises the question; what else could have been done to prevent the development of HBS, and the subsequent 8 week hospital admission? Being vitamin D deficient (treated prior to discharge) may have increased the risk of HBS, or possibly even prolonged it.

CC2

Hypophosphatasia associated with Acute Meningo-Encephalo-Myelitis

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Background: Hypophosphatasia is generally regarded as a disease of bone and teeth. Lack of Tissue Non-Specific Alkaline Phosphatase (TNAP) leads to an accumulation of inorganic pyrophosphate and the Vitamin B6 metabolite pyridoxal 5'-phosphate (PLP), a reduction in pyridoxic acid (PA) and increased PLP/PA ratio. Vitamin B6 deficiency in the brain impairs synthesis of neurotransmitters, and is a well-recognised cause of neonatal seizures. We have found no previous reports of Acute Meningo-Encephalo-Myelitis as a feature of Hypophosphatasia beyond the neonatal period.

Presenting problem: A 12 year old girl with Acute Meningo-Encephalo-Myelitis was noticed to have persistently low serum alkaline phosphatase activity. She had presented to her local hospital with a 1 week history of fever, drowsiness and difficultly walking. She developed increasing weakness, slurred speech and 2 days later respiratory failure requiring ventilation. Brain MRI and EEG showed signs of Meningo-Encephalo-Myelitis. She was born with a malformation of her left hand but never had dental or bone features of hypophosphatasia.

Clinical management: She was treated with intravenous antibiotics, antiviral therapy, steroids and plasmapheresis. It was later noticed that her serum Alkaline Phosphatase activity had been low since presentation (22-37 IU/L). Her plasma PLP was 302 nmol/L (range 20-140) with a PA of 39 nmol/L (9-60) giving a PLP/PA ratio of 8 (normal non-supplemented subjects <5.0) supporting the diagnosis of hypophosphatasia. Genetic analysis showed a pathogenic heterozygous mutation in exon 5 of *ALPL*: c.346G>A, p.Ala116Thr. Review of her neonatal record, and that of her twin sister, revealed that both girls had low alkaline phosphatase activity on routine blood test at 4 days of age (47 and 58 IU/L respectively). The twin has had no symptoms.

Discussion: TNAP is known to be expressed in the synapses of the cerebral cortex that are involved in neurotransmitter synthesis, synaptic stabilization, and myelin pattern formation. This case raises the possibility that that hypophosphatasia might be causally related to Meningo-Encephalo-Myelitis.

LBCC2

A 3-generation family with autosomal dominant hypophosphatasia

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Hypophosphatasia (HPP) is a rare inherited, metabolic disease caused by loss-of-function mutations in the gene encoding tissue non-specific alkaline phosphatase (TNSALP). Inheritance is autosomal dominant or recessive.

Case histories: The proband presented at age 27 with sudden bilateral knee pain and swelling. Three months later, inflammatory arthritis spread to ankles, wrists and elbows, and thigh weakness caused difficulty climbing stairs. There was no history of fractures.

Investigations showed low alkaline phosphatase (ALP) 13-27 U/L (normal range 30-130). Serum pyridoxal 5'-phosphate (PLP) 196 nmol/L (normal range 35-110). Bone density was normal. Nuclear medicine bone scan showed delayed uptake in knees and ankles. Xrays showed no chondrocalcinosis. Calcium pyrophosphate dihydrate crystal deposition disease (CPPD) was diagnosed and she responded to colchicine.

Her mother described joint pain and swelling in a similar distribution since age 20 which responded to colchicine. There was no history of fractures or dental problems. ALP level was 17 U/L and serum PLP was 243 nmol/L.

Genetic testing of the *ALPL* gene identified a familial, heterozygous, pathogenic, missense variant c.[318G>C], p.Gln106His. in exon 5.

Genetic testing confirmed the diagnosis in 3 of the proband's 4 children. The first had delayed primary tooth development (1 tooth at age 1). The second had 12 milk teeth removed due to severe gum disease and developed symmetrical large joint and thigh pain before age 10. The third was unaffected and the 4th was an asymptomatic carrier (2 years).

Discussion: Heterozygote or biallelic mutations in the *ALPL* gene cause deficient TNSALP enzymatic activity resulting in extracellular accumulation of its substrates.

There is wide intra-familial variation in expression and severity. It can present antenatally with severe perinatally lethal form, in childhood or adulthood.

Adults with HPP present with fractures, premature tooth loss, low bone mass, CPPD, or muscle weakness.

Conclusion: In this family with autosomal dominant hypophosphatasia, the predominant features are early onset CPPD and dental problems rather than fractures.

We suspect that this condition is underdiagnosed and should be suspected if the ALP level is low.

LBCC3

A profound lack of Vitamin D: harnessing the potential of genomics

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Background: While most cases of vitamin D deficiency are due to reduced sunlight exposure, rare inherited conditions can also present with rickets and osteomalacia. The advent of genomic sequencing

has the potential to revolutionize our diagnostic and therapeutic approaches in clinical medicine.

Presenting Problem: A 20-year-old woman presented with lifelong refractory vitamin D deficiency and progressive painful scoliosis.

Clinical management: Biochemical testing revealed very low levels of 25OH vitamin D, hypophosphataemia, borderline hypocalcaemia and a high PTH despite therapy with high doses of parent vitamin D, alfacalcidol, calcitriol and/or calcium supplements. An expert panel suggested the use of phosphate supplements which led to spontaneous fractures and worsening symptoms. Whole exome sequencing identified a novel mutation in the vitamin D pathway that informed a successful change in the therapy with a dramatic improvement in the clinical presentation.

Discussion: Genome sequencing provided a fundamental step change in our ability to diagnose and manage this patient. The current 100,000 genomes project provides this resource for many rare bone diseases but is underused. More work is needed to integrate genomic technologies into usual clinical care.

Posters

P1

ARQ-197, a small-molecule inhibitor of c-Met, reduces tumour burden and prevents tumour-associated bone disease in a murine model of myeloma

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Objectives: The receptor tyrosine kinase c-Met, its ligand HGF, and their signalling pathway, have all been implicated in the pathogenesis of myeloma. In myeloma patients with elevated levels of HGF their prognosis is known to be poor. Therefore, targeting these molecules or their pathway in such patients may be of great benefit. We hypothesised that ARQ-197 (Tivantinib), a small molecule c-Met inhibitor, would reduce myeloma cell growth and prevent myeloma-associated bone disease in a murine model.

Methods: In vitro we assessed the effects of ARQ-197 (0.1563 μM - 5 μM) on myeloma cell proliferation, cytotoxicity and c-Met protein expression in the JJN3 human cell line. In vivo we intravenously injected NOD/SCID-γ mice with 10^6 JJN3 cells and 1 week later treated mice with either ARQ-197 (200 mg/kg/day, 5 times per week by oral gavage) or vehicle for 2 weeks.

Results: In vitro exposure of JJN3 cells to ARQ-197 (0.625 μM - 5 μM) resulted in a significant inhibition of cell proliferation ($p<0.0001$) and an induction of cell death ($p<0.001$), probably caused by reduced levels of phosphorylated c-Met. In vivo ARQ-197 treatment of JJN3 tumour-bearing mice resulted in a significant reduction in tumour burden ($p<0.001$), where tumour infiltration of the bone marrow was reduced by approximately 43% (96±4.9% vehicle vs 55±20% ARQ-197 treatment). ARQ-197 treatment also significantly prevented the formation of myeloma-induced bone lesions ($P<0.001$) and the loss of trabecular bone ($p<0.01$) compared to vehicle treated JJN3-tumour bearing mice. Dynamic histomorphometry showed ARQ-197 treatment prevented significant decreases in the mineralising bone surface ($p<0.001$), the mineral apposition rate ($p<0.01$), the bone formation rate ($p<0.01$), and prevented complete loss of osteoblasts on the cortico-endosteal bone surface compared to the vehicle group.

Conclusion: In summary, these results suggest that ARQ-197 could be a promising therapeutic in myeloma patients who express high levels of HGF, leading to both a reduction in tumour burden and an inhibition of myeloma-induced bone disease.

P2

Prevalence of low alkaline phosphatase and *ALPL* mutations in patients with osteoporosis

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Hypophosphatasia is a rare inherited skeletal disorder characterised by defective mineralisation, and multiple fractures caused by loss-of-function mutations in *ALPL* gene. Biochemical features include low levels of serum alkaline phosphatase (ALP) and raised levels of pyridoxal-5-phosphate and phosphoethanolamine. Previous population based studies have estimated that about 1% of individuals have low ALP levels of unknown cause. However the prevalence and clinical significance of low ALP levels in patients with osteoporosis has not been studied. Here we evaluated the prevalence of low ALP levels in a large clinic based population of patients and screened for the presence of *ALPL* mutations in the subjects compared with controls. From a total cohort of 3,285 patients referred to the osteoporosis clinic over a 12 year period we identified 17 (0.52%) in whom ALP levels were low (<40U/l) on at least two occasions. We selected 30 osteoporotic patients from the same population with normal levels of ALP as controls. Sequencing of the ALPL gene was performed in both groups. Mutations in *ALPL* were identified in 13/17 patients with low ALP and 0/30 controls ($p=0.017$). Twelve patients were heterozygous carriers of missense mutations and one had a nonsense mutation (c.303c>a, p.Tyr301X). Of the mutations detected, 8 had previously been reported as causing recessive Hypophosphatasia, but two were novel (c.455g>a, p.Arg152His; c.901a>t, p.Arg301Trp). In silico analysis predicted that p.Arg301Trp is a pathogenic change, whilst inconclusive results were obtained for p.Arg152His. Regression analysis showed that ALPL mutation carriers showed a significant reduced number of comorbidities than controls ($p=0.001$, beta=0.476, 95%CI=[0.28-0.67], $r^2=23\%$). In summary, low serum ALP is unusual in osteoporotic patients but a high proportion of patients who have low ALP are carriers of mutations in *ALPL*. Further studies need to be conducted to address the role of *ALPL* mutations in osteoporosis.

P3 - Abstract withdrawn

P4

Susceptibility genes for hip osteoarthritis may influence hip shape

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Objective: Genome-wide association studies (GWAS) of patients with total hip replacements have identified several OA susceptibility genes. To investigate whether these genes might act by altering hip shape, we examined their associations with hip shape changes previously implicated in the pathogenesis of hip osteoarthritis, including cam-type deformity contributing to femoro-acetabular impingement (FAI).

Methods: 24 loci associated with hip osteoarthritis in previous GWAS were identified by literature search, following which 16,149 single nucleotide polymorphisms (SNPs; >1% allele frequency) within the attendant genes were selected. Hip DEXA scans were obtained from mothers of the Avon Longitudinal Study of Parents and Children (mean 48 years). Hip shape was measured using SHAPE software from the University of Aberdeen; the femoral head and superior acetabulum were outlined, following which 10 independent hip shape modes (HSM) were generated by statistical shape modelling. Three sub-models (each with five further shape modes) were also analysed, designed to capture femoral head sphericity, cam deformity and superior joint space narrowing. Genetic associations were analysed between SNPs selected as above, and each hip shape mode, applying $P<5x10^{-4}$ as the Bonferroni-corrected threshold.

Results: Genetic association analyses, based on 3111 participants, identified 159 SNPs associated with hip shape modes at $P<5x10^{-4}$, located across 12 different genes. For example, in the whole shape model, *MCF2L* (rs116980211) was associated with hip shape mode 3, with the rarer allele positively related to cam-type deformity (β=-0.35, $P=1.8x10^{-6}$). In the sub-models, *TP63* (rs79952512) was related to femoral head mode 4, the rarer allele showing a more ellipsoid femoral head (β=-0.23, $P=1.26x10^{-5}$); *ADGRV1* (rs148878310) was associated with cam-type deformity mode 2, the rarer allele showing greater cam-type deformity (β=0.37, $P=1.1x10^{-4}$); *NOS1AP* (rs73021425; β=-0.13, $P=2.0x10^{-4}$) was associated with superior joint space width mode 3, the rare allele showing a narrower joint space.

Conclusion: Our results suggest that OA susceptibility genes are associated with alterations in hip shape, including cam-type deformity and a change from a spherical to more ellipsoid femoral head shape, which may contribute towards their tendency to increase hip OA risk.

P5

SirT1-deficient intervertebral discs display degenerative morphology and decreased size in vivo

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Ageing is universally linked to musculoskeletal degeneration. Over time, intervertebral discs (IVD) begin to shrink and lose integrity, flexibility, elasticity, and shock-absorbing characteristics leading to pain, numbness or difficulty walking. In some cases, IVDs may collapse completely causing vertebrae to rub against one another. However, despite MRI studies indicating that almost everyone over 60 has some degree of IVD degeneration, not all cause damage and pain, suggesting that a variety of contributing factors might underlie the degree of IVD decline. SirT1 is a well-conserved longevity-linked factor known to control lifespan. Loss of SirT1 expression occurs in ageing human tissues, and leads to accelerated development of many age-related disorders such as bone loss, osteoarthritis. Importantly, levels of SirT1 have recently been shown to decline in ageing human IVD also, and activation of SirT1 in nucleus pulposus (NP) cells and cartilage end plate (CEP) cells *in vitro* promoted cell growth and reduced apoptosis. To investigate whether the direct loss of SirT1 expression predisposes to IVD degeneration *in vivo*, we generated and analysed mice deficient in SirT1 within IVDs.

IVD-conditional SirT1 knockout mice were generated by crossing $SirT1^{fl/fl}$ mice with transgenic mice expressing Cre recombinase within the Aggrecan promoter region. Aggrecan-Cre expression within the IVD region was confirmed by breeding with ROSA26R mice followed by β-gal staining of E15.5 embryos. IVDs from decalcified vertebrae of knockout mice ($SirT1^{Agg}$) or controls ($SirT1^{fl/fl}$)(n=6) were analysed by histological (following H&E, Toluidine blue, Congo Red, PAS, D/PAS, Goldner's trichrome staining) and histomorphometric analysis.

IVD from $SirT1^{Agg}$ mice showed increased compression of the NP region (282.8μm±20.9 Vs 376.2μm ±20.1, $p<0.01$) and between CEP of adjacent vertebral bodies (452.2μm±16.5 Vs 567.6μm±18.8, $p<0.001$) compared to $SirT1^{fl/fl}$ control mice. Morphologically, tinctorial staining of IVD sections revealed a less organised arrangement of connective tissue fibres and chondrocytes within the annulus fibrosis and CEP regions respectively, in $SirT1^{Agg}$ mice versus controls.

Our data suggests that loss of SirT1, as seen in ageing human tissues, dysregulates the normal structure of the IVD and may predispose to disc degeneration.

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P6

Relationships between DNA methylation, femoral neck bone mineral density and grip strength from an epigenome wide association study: the Hertfordshire Cohort

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Objectives: We investigated epigenome-wide DNA methylation in the peripheral blood of older adults in relation to bone indices and grip strength.

Methods: Femoral neck (FN) bone mineral density (BMD) was assessed using DXA (Hologic QDR 4500) in 49 men and 50 women from the Hertfordshire Cohort Study (mean age 64.4 years). Grip strength was assessed using a Jamar dynamometer. DNA methylation in these subjects was analysed using the Infinium HumanMethylation450 BeadChip (450k). Standard quality control and removal of ambiguous and co-locating SNP probes resulted in the assessment of 383,230 CpGs. The results were adjusted for age, sex, plate position, chip, and white blood cell composition.

Results: We found differentially methylated positions (DMPs) at epigenome-wide statistical significance (using the Bonferroni

correction, p-value ≤1.305×10^{-7}), including 10 DMPs with FN BMD and 36 DMPs with grip strength. Of note was the identification of DNA methylation changes associated with both femoral neck BMD and grip strength in *DTWD2*, a gene previously associated with BMI and subcutaneous adiposity, and *SKI*, a nuclear proto-oncogene associated with skeletal, muscular and arterial morphology. Of interest, the DMP within *SKI* aligns with a transcription factor binding site in human umbilical vein endothelial cells. A monogenic disorder, Shprintzen-Goldberg craniosynostosis syndrome, which has a skeletal phenotype similar to Marfan's syndrome, is caused by heterozygous mutations in the *SKI* gene. KEGG pathway analysis (FDR≤0.20, and corrected for array bias with missMethyl) for determinants of FN BMD was enriched for differentially methylated genes associated oestrogen signalling, thyroid hormone signalling and in signalling pathways regulating the pluripotency of stem cells.

Conclusion: We identified a set of differentially methylated genes involved in skeletal, muscular, connective tissue, adiposity and vascular function that were related to musculoskeletal outcomes in later adulthood. Our findings potentially indicate tissue-independent epigenetic mechanisms acting throughout the lifecourse in the pathogenesis of poorer bone health in older age, or the identification of passive biomarkers of this process. Replication in a separate cohort will be required to confirm these results.

P7

Valve interstitial cell (VIC)-derived matrix vesicles (MVs) regulate the pathogenesis of Calcific Aortic Valve Disease (CAVD): a role for Annexin-VI?

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Objectives: End-stage renal disease (ESRD) patients have elevated circulating calcium (Ca) and phosphate (Pi), and exhibit accelerated progression of calcific aortic valve disease (CAVD). Past studies revealed that vascular calcification share many similar features with bone formation, including the secretion of matrix vesicles (MVs), which are believed to initiate mineralisation. This study aims to unravel the role of MVs during aortic valve calcification.

Methods: Primary rat valve interstitial cells (VICs) were cultured in either control or calcifying condition (2.7 mM Ca and 2.5 mM Pi) for 3 to 5 d to induce calcification. MVs were harvested from rat VICs using differential centrifugation and their content was analysed using iTRAQ mass spectrometry. Further bioinformatics analysis of the results was conducted using Ingenuity Pathway Analysis (IPA). Human aortic valve samples were analysed using immunohistochemistry and MVs were located using immunogold staining coupled to transmission electron microscopy (TEM).

Results: Cultures under calcifying condition (2.7 mM Ca and 2.5 mM Pi) induced calcium deposition (10.8 fold compared to control cultures; p<0.001) in VICs. Furthermore, elevated Ca and Pi treatment increased the mRNA expression of the osteogenic markers *Msx2, Runx2* and *Alpl* (p<0.01).

Proteomics analysis of rat VIC-derived MVs revealed the marked enrichment of established exosomal proteins, including CD9, CD63, LAMP-1 and LAMP-2 and a concomitant up-regulation of the Annexin family of calcium-binding proteins. Of particular note Annexin VI was shown to be enriched in calcifying VIC-derived MVs (51.9 fold; p<0.05). Subsequent IPA analysis showed the up-regulation of notable canonical signalling pathways relevant to cardiovascular function including aldosterone, Rho kinase and metal binding.

TEM analysis of human calcified valve tissue revealed the presence of MVs in the extracellular matrix and the expression of Annexin VI was upregulated in areas of MVs.

Conclusions: These findings highlight a critical role for VIC-derived MVs in CAVD. Furthermore, we identify calcium as a key driver of aortic valve calcification, which may underpin the increased susceptibility of ESRD patients to accelerated development of CAVD.

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P8

The effect of social deprivation on hip fracture incidence in men and women over 14 years across regions in England

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Social deprivation predicts a range of adverse health outcomes, including hip fracture, though whether inequalities in incidence rates have lessened over time is unknown. We examined the effect of area level social deprivation on hip fracture incidence in England, and its regions, over 14 years.

We used English Hospital Episodes Statistics (2001/02-2014/15) to identify index hip fractures (ICD-10 codes S72.0/S72.1/S72.2) among English residents aged 50 or older and mid-year population estimates (2001-2014) from the Office for National Statistics. The Index of Multiple Deprivation (2010) was used to measure deprivation in quintiles (Q1 least deprived; Q5 most deprived). We calculated incidence rate ratios (IRR) for hip fracture, adjusting for age, stratified by deprivation status, gender and region.

We identified 747,369 hospital admissions with an index hip fracture over 14 years. Median age was 83 years (interquartile range 77-88); 74.2% of fractures occurred in women. Whilst incidence rates decreased in women (annual reduction 1.1% per year), they increased in men (annual increase 0.6% per year) (interaction p-value <0.001).

In men, the incidence rate ratio for most versus least deprived was 1.35 [95%CI 1.33,1.37]; a less marked relationship was observed in women (IRR 1.06 [1.05,1.07], (p-value for interaction p<0.001). Age-standardised incidence increased similarly for men across all deprivation strata from 2001 to 2014, but fell to a greater extent amongst women in those least deprived compared to most deprived (interaction p-value <0.001).

The effect of deprivation on hip fracture incidence differed by region for men and women (interaction p-value <0.001). Hip fracture incidence was highest among the most deprived in the North of England (females: Q1 vs. Q5 213 [210, 216] fractures/100,000 vs. 684 [678, 690] fractures/100,000), whilst the opposite trend was seen in the South of England, with no clear pattern in the Midlands.

Deprivation is a stronger predictor of hip fracture incidence for men

than women and was most marked in the North of England. The inequality gradient for hip fracture remains unchanged for men and has actually widened for women.

P9

Effect of applied mechanical loading on skeletal bone blood flow in mice

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Angiogenesis and increased skeletal perfusion are essential for bone formation. While mechanical loading of bone is known to induce osteogenesis, it is unknown whether skeletal blood flow is increased by applied mechanical loading to bone and could contribute to enhanced bone formation. The aim of this study was to investigate if there is an association between load-induced osteogenesis and bone blood perfusion. Mechanical loading was applied to the right tibiae of male C57BL6 mice aged 10-12 weeks through the knee joint for two weeks, 3 times/week at 12N. Bone blood flow was measured in the hind-limb using Laser Doppler imaging both acutely, immediately after each episode of loading and chronically after 2 weeks of mechanical loading. Blood flow was compared to the non-loaded contralateral hind-limb. Bone formation was assessed in trabecular and cortical compartments using micro-CT analysis. Before the first loading episode, measurements of the mean perfusion ratio between loaded and non-loaded hind-limbs demonstrate similar perfusion in both legs. The first episode of mechanical loading acutely increased the mean flux ratio between loaded and non-loaded limb by 56% compared to the baseline ($P<0.01$ vs baseline). Moreover, these acute load-induced increases in blood flow were conserved since the mean flux ratio was also acutely elevated by 50% (compared to the baseline; $P<0.001$) immediately after the 6th episode of mechanical loading. In contrast, two weeks of loading failed to engender any chronic changes in mean perfusion ratio between the beginning and the end of the experiment, suggesting that the basal tone is not changed by two weeks of applied mechanical loading. As expected, mechanical loading increased both trabecular and cortical bone volumes and architectures in the loaded compared to the contralateral control tibiae, indicating new bone formation in the loaded limb. Our results indicate that acute and conserved increases in bone perfusion, but not chronic modifications in basal vascular tone are linked with the new bone formation induced by mechanical loading.

P10

Association between physical activity and scoliosis: A prospective cohort study

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Objectives: Scoliosis is a three-dimensional torsional rotation of the spine. The most common sub-type is Adolescent Idiopathic Scoliosis (AIS). Because of lack of population-based studies, little is known about the determinants of initiation of scoliosis. The link between physical activity (PA) and scoliosis is of great potential interest. The aim of this study was to carry out the first population-based prospective analysis of the association between PA in early life and onset of AIS by aged 15 years.

Methods: The Avon Longitudinal Study of Parents and Children (ALSPAC) is a population-based cohort. Data on mother-reported PA were collected at aged 18months, 4.5 years and 9.9 years. Objectively measured PA data were collected at aged 11years by an Actigraph accelerometer. Data were collected on scoliosis at aged 9 and 15 years. Associations between PA variables and scoliosis were examined by logistic regression before and after adjustment for confounders.

Results: Of 4640 adolescents, 267 (5.8%) had developed scoliosis between aged 9 and 15 years. After adjustment for confounders, higher levels of mother-reported PA at aged 18 months and 9.9years were associated with a reduced risk of scoliosis at aged 15 years (OR for scoliosis with higher PA at 18 months of 0.34, 95%CI 0.13 to 0.90, P=0.030; OR for scoliosis with higher PA at aged 9.9 years of 0.67, 95%CI 0.51 to 0.88, P=0.004). Similarly, higher levels of objectively measured accelerometry data at aged 11 years were associated with reduced risk of scoliosis at aged 15 years (OR for scoliosis per SD increase in moderate/vigorous PA of 0.69, 95%CI 0.59 to 0.82, $P<0.001$). Mother-reported PA at aged 4.5years showed a similar direction of association but did not reach statistical significance.

Conclusions: Children who do more PA are less likely to develop scoliosis. This may indicate that lower PA is a novel risk factor for scoliosis initiation, perhaps because the scoliosis deformity occurs as a result of reduced loading. Alternatively, the lower PA is an early manifestation of the underlying abnormality that eventually results in AIS.

P11

Vascular calcification and bone formation: are they the same?

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Vascular calcification is the deposition of calcium phosphate mineral, often as hydroxyapatite, in the arteries. It shares superficial similarities to skeletal mineralisation and has been associated with the transdifferentiation of vascular smooth muscle cells (VSMCs) to an osteoblast-like phenotype. This investigation used mouse VSMCs and osteoblasts to directly compare *in vitro* vascular calcification and bone formation. Control VSMCs (no added phosphate), calcifying VSMCs (+2mM phosphate) and osteoblasts (2mM β-glycerophosphate) were cultured ≤14 days, and changes in cell differentiation, survival, function and gene expression assessed. Osteoblasts formed large mineralised bone nodules that were associated with widespread deposition of collagenous extracellular matrix. In contrast, VSMCs formed small discrete regions of calcification that were not associated with matrix deposition and did not resemble bone. Calcifying VSMCs displayed progressive reduction in cell viability over time (≤7-fold, $p<0.001$), with a 50% increase in apoptosis ($p<0.001$); the viability of mineralising osteoblast cultures and control VSMCs remained unchanged. Alkaline phosphatase (TNAP) activity in VSMCs was ~100-fold lower than that of mineralising osteoblasts ($p<0.001$), although it increased by 50% ($p<0.001$) in calcifying cultures. Moreover, no calcification was observed in VSMC cultures treated with β-glycerophosphate, a TNAP substrate. Ecto-nucleotidase pyrophosphatase/phosphodiesterases (NPPs) generate the ubiquitous calcification inhibitor, pyrophosphate from extracellular ATP. Culture in phosphate-supplemented medium was associated with a 3-fold reduction in VSMC NPP activity

(p<0.001), compared with controls. Nonetheless, calcifying VSMCs still displayed 3-fold higher levels of NPP activity than mineralising osteoblasts (p<0.001). Calcifying (day 14) VSMCs displayed <3-fold increases in expression of the osteoblast marker genes osteocalcin and TNAP, compared with day 7 cultures; in contrast, expression of the same genes increased ≤7-fold in osteoblasts over the same time period as they differentiated into the bone-forming phenotype. In summary, calcifying VSMCs display some limited osteoblast-like characteristics but also differ in several key respects: 1) their inability to form collagen-containing bone; 2) their lack of reliance on TNAP to promote mineral deposition; 3) the deleterious effect of calcification on their viability. Extracellular ATP effectively inhibits calcification in VSMCs, suggesting an important regulatory role for the NPP - pyrophosphate system.

P12

Muscle strength and physical performance from midlife and bone health in early old-age: the MRC National Survey of Health and Development

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There are few prospective data investigating how muscle strength and physical performance in adulthood may relate to later bone health. The aim of this study was to test associations between grip strength, standing balance and chair rise speed, measured from midlife, with bone outcomes in early old age in men and women from a British birth cohort study, the MRC National Survey for Health and Development.

At age 60-64y, 1583 (824 women) had assessments of bone including pQCT (radius) and DXA (hip). Analyses were stratified by sex, and associations between bone outcomes (radius: volumetric BMD, cross-sectional area (CSA), medullary area; hip: aBMD and CSA, femoral neck cross-sectional moment of inertia (CSMI)) and grip strength, chair rise speed and standing balance (assessed at ages 53 and 60-64) were tested using linear regression before and after adjustment for height and weight. Results are presented as percentage mean [95%CI] difference in DXA- and pQCT outcome per unit difference in strength or performance measure.

In men, stronger grip at ages 53 and 60-64 was associated with greater radius (0.3 [0.2,0.4]) and hip (0.2[0.2,0.3]) CSA, femoral neck CSMI (0.5[0.3,0.6]) and hip aBMD (0.2[0.1,0.3])); all p<0.001. Associations with CSA and CSMI measures were robust to adjustment. In women, similar sized associations were found and remained after adjustment for radius (0.4[0.2,0.5]) and hip (0.2[0.1,0.3]) CSA and hip aBMD (0.2[0.0,0.3]). Neither chair rise speed nor standing balance were consistently associated with bone outcomes.

Grip strength had the most consistent, positive associations with clinically relevant bone outcomes at the radius and hip. Fewer associations were found with chair rise and standing balance performance, possibly because grip strength is a more direct measure of muscle function and therefore better indicator of the muscle bone relationship than these measures which are dependent on more body systems. Evidence for the importance of muscle bone relationships in midlife and early old age suggest midlife may provide an opportune time for intervention for prevention of disability associated with sarcopenia and osteoporosis in later life.

P13

Deciphering the signalling mechanisms underpinning fibroblast growth factor-2 (FGF-2) mediated osteocytogenesis

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Objectives: Our previous work has shown that fibroblast growth factor-2 (FGF-2) stimulates E11 expression and hence promotes the acquisition of the osteocyte dendritic phenotype. Here we aimed to decipher the intracellular signalling pathways underlying FGF-2's stimulation of E11 expression.

Methods: MC3T3 clone-14 osteoblast-like cells were challenged with recombinant murine FGF-2 (10 ng/ml) for various durations (5 mins - 48 hrs) and the expression of total and phosphorylated intracellular signalling molecules, E11 and fibroblast growth factor receptors (FGFRs) determined. Inhibitors to phosphorylated (p-) ERK (U0126), Akt (LY294002) and FGFRs (AZD4547) were used to evaluate the relative importance of specific signalling pathways in FGF-2 mediated E11 stimulation.

Results: FGF-2 treated cells disclosed a significant increase in p-p44/42 (ERK) compared to controls (P<0.001; relative toP44/42/βactin). Smaller, but significant increases were also observed in p-Akt (P<0.01; relative to Akt/βactin) and p-p38 (P<0.05; relative toP38/βactin). No significant increase in JNK activity by FGF-2 was noted. Treatment of cells with FGF-2 for up to 48hrs revealed sustained activation of p-ERK, suggesting that this is critical for osteoblast-osteocyte differentiation. Inhibition of p-ERK at high concentrations (≥25 μM) of U0126 had no effect on E11 expression, possibly due to an observed compensatory increase in p-Akt activation. However, dual inhibition of ERK and Akt signalling via U0126 (25 μM) and LY294002 (10 μM) also did not reduce E11 expression to basal levels after 4 and 24hrs of FGF-2 stimulation. RT-qPCR revealed that MC3T3 cells expressed *Fgfr1/2/3*, but not *Fgfr4*, with *Fgfr1* expression 15-fold more highly expressed than *Fgfr2/3*. This indicates that the effects of FGF-2 may be mediated principally through the activation of *Fgfr1* in MC3T3 osteoblast-like cells. FGF-2 decreased the expression of $^{Fgfr2/3}$ but not *Fgfr1* after 4 and 24hrs challenge. AZD4547 (100-1000 nM) inhibited the expression of p-ERK after 15 mins of FGF-2 exposure.

Conclusion: FGF-2 stimulates persistent ERK activation in osteoblast-like cells but our inhibitor studies suggest that additional signalling pathways may mediate its down-stream stimulation of E11 expression and acquisition of the osteocyte phenotype.

P14

25-hydroxyvitamin D response to cholecalciferol supplementation in pregnancy is associated with common vitamin D related genetic variants: findings from the MAVIDOS trial

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Objectives: Single nucleotide polymorphisms (SNP) in genes related to vitamin D metabolism have been associated with 25-hydroxyvitamin D [25(OH)D] status, but these relationships have not been examined in pregnancy or following antenatal vitamin D supplementation. We assessed whether SNPs in *DHCR7* (7-dehydrocholesterol reductase), *CYP2R1* (25-hydroxylase), *CYP24A1* (24-hydroxylase) and GC (Vitamin D binding protein) were associated with the response to vitamin D supplementation in pregnancy.

Methods: MAVIDOS is a randomised double-blind placebo-controlled trial of 1000IU/day cholecalciferol from 14 weeks gestation until delivery in women with a baseline 25(OH)D of 25-100 nmol/l. Anthropometry, serum 25(OH)D (Diasorin Liaison), health and diet were assessed at 14 and 34 weeks gestation. Genotyping using KASP™ competitive allele-specific PCR (LGC Genomics, Hoddeston, UK) included rs12785878 (*DHCR7*), rs10741657 (*CYP2R1*), rs6013897 (*CYP24A1*) and rs2282679 (*GC*). Multiple linear regression was performed using an additive model with the homozygous minor allele as the reference group (beta represents the change in outcome per additional major allele), adjusting for a number of previously identified determinants of 25(OH)D.

Results: 712 women (367 placebo, 345 cholecalciferol) were included (95.8% White ethnicity). Only rs12785878 (*DHCR7*) was associated with baseline 25(OH)D [β=4.1 nmol/l (95% CI 2.2, 6.1), p<0.001]. Conversely in women randomised to the cholecalciferol supplement, rs10741657 (CYP2R1) [β=-4.1 nmol/l (95%CI -7.1, -1.2), p=0.006] and rs2282679 (GC) [β=4.4 nmol/l (95%CI 1.2, 7.6), p=0.007] were associated with achieved 25(OH)D at 34 weeks gestation, but rs12785878 (GC) and rs6013897 (*CYP24A1*) were not. A genotype risk score including rs10741657 and rs2282679 was negatively associated with late pregnancy serum 25(OH)D concentration after supplementation, such that for each additional risk allele 25(OH)D was reduced by 4.0 nmol/l (95% CI 1.9, 6.2 nmol/l, p<0.001).

Conclusion: Genetic variation in *DHCR7*, which encodes 7-dehyrocholesterol reductase in the cholesterol/vitamin D biosynthesis pathway in the skin appears to modify baseline 25(OH)D, whereas the response to antenatal cholecalciferol supplementation was associated with SNPs in *CYP2R1* and *GC*. These may may alter 25-hydroxylase activity and vitamin D binding protein synthesis, respectively. Women with more "at risk" alleles may require higher supplement doses to achieve vitamin D repletion in pregnancy.

P15

Evidence for gender-specific vascular signalling in bone

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Until recently, most gender differences in bone disease were attributed to circulating levels of oestrogen and testosterone. Evidence now suggests that sexual dimorphism depends on fundamental differences at the genetic level. Low concentrations of pro-angiogenic vascular endothelial growth factor (VEGF) have been found in postmenopausal women. The role of VEGF in male bone homeostasis is unknown and was the focus of this study. We investigated the influence of *in vivo* osteoblast (OB) VEGF deletion on intracortical bone structure in male versus female mice, in addition to investigating sex-specific OB communication with endothelial cells (ECs) *in vitro*.

For VEGF knock out (KO) in OB cells 16-week-old male and female mice carrying floxed alleles of VEGF and expressing Cre-recombinase under the Osteocalcin promoter (Ocn) were compared. Synchrotron computed tomography was used to disclose the intracortical porosity at the tibia-fibula junction. OBs were extracted from male and female neonates and cultured separately. To assess indirect effects of VEGF on osteogenesis via the vasculature, conditioned media (CM) from VEGF KO male and female OBs and wild type (WT) cells were added to mouse bone marrow EC cultures for gene expression.

In male versus female WT animals no difference in intracortical porosity was observed (p=0.1215), however following VEGF-deletion, the cortical bone of male KO animals was 179% more porous than female KO animals (±34.2%, p=0.0003). VEGF treatment had no direct effect on male or female OB viability. Similarly, VEGF-deletion did not impact OB differentiation or viability. Consistent with an indirect effect of VEGF in bone existing via the vasculature, ECs treated with CM from male VEGF KO OBs showed decreased levels of Insulin-like growth factor-1 (IGF-1) expression (-2.33 fold) in contrast to the CM from female VEGF KO OBs, which increased EC IGF-1 mRNA expression (+3.07 fold) versus controls.

The decreased expression of osteogenic genes such as IGF-1 by ECs in the absence of VEGF could contribute to the more severe phenotype identified in male VEGF Ocn KO mice. Targeting vascular signals to modulate bone formation between sexes could provide a more effective way to treat degenerative bone disease.

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P16

Multiple myeloma regulates bone marrow adipocyte number, localisation and adipokine secretion

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Objectives: Multiple myeloma (MM) is a fatal haematological malignancy where tumour growth and bone disease are dependent

upon cellular interactions within the bone marrow. Bone marrow adipocytes (BMAs) have an emerging role in bone physiology and are a major source of adiponectin, an adipokine negatively associated with MM. Our goal was to elucidate the reciprocal relationship between MM cells, adiponectin and BMAs in vitro and in vivo.

Methods: We have combined in vivo studies using the 5TGM1 murine model of MM and imaging of BMAs with in vitro cellular and molecular biology. Studies have used a panel of MM cell lines, BMAs differentiated from ST2 bone marrow stromal cells (BMSCs) and primary MM cells, BMSCs and BMAs derived from patients with MM.

Results: We have examined the number and localisation of BMAs during development of myeloma in vivo, using perilipin to identify BMAs. A significant negative association between tumour burden and BMA number was demonstrated ($p<0.05$). Further analysis revealed a 40% increase in BMAs closely associated with tumour, and an 83% reduction in BMAs in areas of non-tumour bone marrow, suggesting a differential response of BMAs within the myeloma-bone microenvironment. Coculture of MM cells with BMAs or BMSCs increased MM cell viability by up to 95%, induced a 4-fold increase in migration and decreased apoptosis, with no significant difference between BMSCs or BMAs. A significant increase in adiponectin mRNA and protein was detected in BMAs as compared to BMSCs, in both cell lines and primary cells. An adiponectin receptor agonist induced MM cell apoptosis, however coculture of MM cells with BMAs significant decreased adiponectin mRNA expression and protein expression and secretion ($p<0.05$), providing a mechanism by which MM cells can down-regulate adiponectin to avoid the tumour-suppressive effect of this adipokine.

Conclusions: BMAs are closely associated with MM cells in vivo. Our studies suggest a supportive effect of BMAs on MM growth and survival, mediated in part by a reduction in adiponectin. Elucidating the BMA-MM relationship could reveal new therapeutic approaches for the treatment of MM.

P17

Use of hip DXA scans to identify shape changes associated with hip osteoarthritis

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Statistical shape modelling (SSM) has recently been demonstrated to capture hip shape from hip DXA scan images. We investigated whether this approach could be used to evaluate risk of hip osteoarthritis (OA), based on the identification of hip shapes associated with femoro-acetabular impingement (FAI), given its published implication in the pathogenesis of hip OA.

Using hip DXA images from the Osteoporotic Fractures in Men (MrOS) Study cohort, hip shape was measured using SHAPE software; the femoral head and superior acetabulum were outlined, following which independent hip shape modes (HSM) were generated by SSM. Hip OA was determined using Croft scoring of hip radiographs, questionnaires and clinical examination (internal rotation of the hip) of the same individuals. Logistic regression was used to test the association between HSMs and radiographic hip OA (RHOA) and symptomatic hip OA (SHOA) adjusting for height, weight, age and race (standardised coefficients are presented).

HSMs were generated from right hip DXA images from 5,862 individuals, mean age 72.8 years, of whom 4,100 had corresponding hip radiographs taken on average 4.6 years later. Five HSMs were associated with RHOA, all of which showed features of FAI as reflected by either pincer- or cam-type deformities. HSM 1 (increased pincer-type deformity) was positively associated with RHOA [1.23 (1.09,1.39)] [odds ratio and 95% CI]. HSM 8 (reduced pincer-type deformity) was inversely associated with RHOA [0.79 (0.70,0.89)]. HSM 10 (increased cam-type deformity) was positively associated with RHOA [1.21 (1.07,1.37)]. HSM 3 and HSM 4 (reduced cam-type deformity) were inversely associated with RHOA [0.73 (0.65,0.83) and 0.82 (0.73,0.93) respectively]. In addition, HSM 3 was inversely related to pain on examination [0.84 (0.76,0.92)] and walking [0.88, (0.81,0.95)], and to the Western Ontaria and McMaster Univerisities Index (WOMAC) [-0.14 (-0.22, -0.07)] [beta and 95%CI] which are all components of SHOA.

Shape changes related to FAI, identified on hip DXA images, were positively associated with both prevalent RHOA and SHOA. Hence, hip DXA scans might prove useful in identifying those at increased risk of hip OA, based on the identification of shape changes associated with FAI.

P18

18F-FDG PET/CT imaging following insulin treatment and short term cold exposure in mice

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Introduction: Throughout the last decade, developments in whole-systems and murine models have identified the skeleton as an endocrine organ. Within the skeleton resides the marrow adipose tissue (MAT), which may be functionally distinct from both white and brown adipose tissue; however, it is unclear if, like these other adipose depots, MAT contributes to systemic energy homeostasis. A recent study identified the skeleton as a significant site of basal and insulin-stimulated glucose uptake, but the authors did not distinguish between bone and marrow uptake[1]. Such knowledge may reveal the function of MAT in normal physiology and disease.

Objectives: Assess [18F]-FDG uptake into bone and the marrow cavity (MC) following insulin treatment (1) and cold exposure (2).

Methods: Mice were fasted for four hours and then given [18F]-FDG, intraperitoneally, one-hour pre-scanning. Objective 1: fasting mice were housed at room temperature; treated with intraperitoneal insulin (0.75 IU/g) or saline (0.9%) immediately before FDG administration; then housed at room temperature before scanning. Objective 2: mice were housed at room temperature or 4°C during fasting and post-FDG administration. One-hour post-FDG, mice were imaged under isoflurane anaesthesia using a nanoPET/CT scanner. A 30-min whole-body emission scan was followed by CT scanning for attenuation correction and co-registration with PET data. Tissues were harvested and radioactivity assessed using a gamma counter. PET images were analysed using PMOD software. Measured activities of target tissues were expressed as standard uptake values (SUV).

Results: [18F]-FDG uptake in bone was significantly higher than in other classical glucose storage organs including subcutaneous, mesenteric and gonadal adipose tissues. Within bones, the MC appears

to be the predominant site of [18F]-FDG uptake. Insulin stimulated [18F]-FDG uptake in femurs, as previously reported[1]; however insulin did not affect glucose uptake in the MC. It has been suggested that MAT is brown-adipose-tissue-like[2], although our assessment of [18F]-FDG uptake following acute cold exposure demonstrated that neither bone nor the MC cavity was cold responsive.

Conclusion: The MC is a significant site of glucose uptake that does not appear to be regulated by insulin or cold exposure.

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P19

Bone health in boys with Duchenne muscular dystrophy: The dichotomy between bone density and fracture

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Current guidelines recommend annual assessments of bone densitometry in boys with DMD. However, this recommendation is based on the assumption that bone density is a predictor of fractures in this patient group. The aim of this study was to evaluate the relationships between long-term changes in bone density, corticosteroid exposure and mobility with vertebral and long bone fractures.

Twenty-four DMD boys (mean age 10.1(SD2.4) years) with at least 6 annual DXA assessments were included in the study; each boy had 3 measures whilst ambulant and 3 measures once ambulation had ceased. A repeated measures model was used to compare size adjusted lumbar spine BMD (BMAD), total body less head BMD (TBLH BMD), lean body mass (LBM) and corticosteroid (CS) cumulative exposure with fractures and mobility.

Over 5 years, 9 long bone fractures were reported in 8 boys and 41 vertebral fractures in 14 boys, of which 6 and 4 respectively, occurred after loss of ambulation; only 7 boys (29%) remained fracture free. At baseline, no differences were seen between the fracture and non-fracture groups for height, LBM and BMAD. Boys who developed vertebral fractures were heavier (p=0.04) and had a higher CS exposure (p=0.02) whilst those who developed long-bone fractures were lighter (p=0.04) and had lower TBLH BMD (p=0.05). BMAD, TBLH BMD, & LBM Z-scores declined consistently over the measurement time frame but the rate of decline was greatest once ambulation ceased (p<0.001). There was a significant positive interaction between CS exposure and vertebral fracture but this was not seen in those who developed long bone fractures.

The only distinguisher of long-bone fractures was low TBLH BMD whereas vertebral fractures were not associated with low BMAD, TBLH BMD or rate of loss of bone density. Cumulative CS exposure was associated with vertebral fractures but not long-bone fractures. Both fracture types were more likely after loss of mobility. This dichotomy between bone density as assessed by DXA and fractures may be potentially misleading when monitoring bone health in boys with DMD. Current guidelines should be revised to reflect these issues.

P20

Bisphosphonates regulate autophagy *in vitro* and *in vivo*

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For decades, the bisphosphonate (BP) class of drug has been the front line treatment for age-related bone loss. However in recent years, BPs have shown wider beneficial effects in humans including anti-cancer properties, increased longevity. One such ageing-related mechanism, Autophagy, involves the identification and breakdown of damaged or defective proteins and recycling of useful base material for continued protein production. Impaired autophagy is associated with many age-related disorders (e.g. diabetes, neurodegeneration) where unwanted proteins accumulate to impair cellular function. Therefore, we have explored whether BPs might control autophagy *in vitro* and *in vivo*, to investigate whether the reported effects of BP to increase lifespan might be mediated by preventing the age-related decline in the autophagic process.

Alterations in autophagy can be assessed by quantifying the crucial autophagy-related protein LC3, and its conversion from LC3-I to LC3-II as the protein-degrading autophagosomme structure is formed. Bone stromal cells (ST2) were treated with zoledronic acid (ZOL), risedronate (RIS) or novel BP derivative OX14 at 0.5-100uM for 4, 24 or 48 hrs and BP activity confirmed by assessing the prenylation status of the Rap1a protein. Also,7-8 week old female C57BL6 mice were injected s.c. 2/week with either ZOL (125 µg/kg), RIS (125 µg/kg), OX14 (1.25, 12.5 or 125 µg/kg) or PBS control (100 ul/mouse). After 21 days, bone and soft tissue samples (heart, gut, kidney, spleen, liver) were collected, and BP activity and autophagy assessed in protein lysates by western blot as above.

Treatment with ZOL, RIS and OX14 increased Rap1a prenylation *in vitro* (up to 18-fold in RIS (20uM) Vs vehicle), which corresponded with a stimulation in autophagic flux in these cells, evidenced by increased LC3-II/LC3-I ratio. Similarly, positive changes in autophagy were seen in heart, gut and liver tissues *in vivo* following BP treatment, particularly OX14 (125 ug/kg) where a 3-fold increase in LC3-II/I ratio was observed.

Our data suggests that BPs can activate autophagy in bone stromal cells in vitro and at certain soft tissue sites when delivered in vivo, indicating that BP treatment might prolong healthspan and lifespan by protecting against an age-related decline in autophagy.

P21

Extracellular vesicles-derived from mineralising osteoblasts induce stem cell osteogenesis: a new phase in regenerative medicine?

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Introduction: Over the last decade, considerable attention has been focussed on developing cell-based approaches for bone regeneration. Although these methods have yielded promising results, their translation is frequently hindered by insurmountable regulatory hurdles. We sought to determine whether extracellular vesicles

(EVs) naturally generated by mineralising osteoblasts could be administered to induce mesenchymal stem cell (MSC) osteogenesis. We anticipate that the use of EVs for bone regeneration may have many advantages over cell-based approaches, providing an acellular yet biological means for regenerating hard tissues.

Methods: EVs were isolated from mineralising murine osteoblasts using differential centrifugation and profiled using atomic force microscopy, direct light scattering and transmission electron microscopy (TEM). Their effects on MSC osteogenic differentiation were assessed against the clinical gold-standard, BMP-2, in the presence and absence of mineralisation medium (MM). MSC osteogenesis was analysed using alizarin red calcium staining and alkaline phosphatase (ALP) quantification. Mineral phase and quality was determined using X-ray fluorescence (XRF) and infrared spectroscopy (IR). LC-MS/MS was used to define the EV proteome and raw data files processed using MaxQuant. MS/MS spectra were searched against the mouse proteome and analysed using Gene Ontology Enrichment analysis.

Results: When added in the presence of MM, EVs of ~160 nm were found to significantly enhance ALP levels, mineralisation rate and mineral volume beyond the current gold-standard. TEM appeared to indicate areas of electron-dense EV-membrane-associated mineral condensation. MSC mineralisation was only significantly enhanced when EVs were administered in the presence if MM. Alizarin red staining and XRF elemental mapping of mineral deposited within MSC cultures identified comparatively large areas of calcium deposition following treatment with EV/MM. IR spectroscopy confirmed the presence of amide peaks corresponding to extracellular collagen. LC-MS/MS proteomic analysis of EVs revealed the temporal deposition bridging collagens (types-XII and VI) essential for osteoblast communication. EVs were also enriched for calcium/phospholipid binding proteins (annexins) and osteopontin.

Conclusion: Our data suggests that EVs function to enhance MSCs capacity to utilise exogenous phosphates. As such, they hold considerable potential as an acellular yet biological approach to regenerative medicine.

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P22

T Score correlation with micro CT measurements of the femoral head in hip fracture

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Objectives: A region of focal osteoporosis has been demonstrated in femoral neck fracture patients[1]. This structural deficit is thought to play an important role in fracture initiation during a fall to the side. We examined the femoral head and a core of bone taken from the focally osteoporotic region of the femoral head: we used micro CT to measured bone volume (BV/TV). These measurements were compared with the T Scores taken from DEXA-like measurements of in vivo CT scans. We hypothesized that standard bone mineral density T scores measured in 2D would correlate with focal osteoporosis measured in critical zones of the femur.

Methods: We performed hip CT on patients who had just sustained a hip fracture, then high resolution CT of the femoral head removed at surgery, followed by ultra-high resolution CT of cores from the region of focal osteoporosis. DEXA-like measurements were taken from the CT scan of the intact hip and T scores calculated. Measurements of BV/TV on the femoral heads and cores were made using ImageJ/BoneJ[2]. Results were plotted in Excel.

Results: There was a range of T scores not all of them osteoporotic (mean -2.02 IQR -1.4 - -2.54).There was strong correlation between femoral neck T score and bone volume in the femoral head as a whole but no correlation between femoral neck T Score and bone volume in the first hundred trabecular slices of the core taken from the region of focal osteoporosis.

Conclusions: We conclude that femoral neck T score correlates well with micro CT measurements of the femoral head in hip fracture patients regardless of osteoporotic status. However standard 2D hip imaging for fracture prediction cannot detect focal osteoporosis.

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P23

A mammalian transcriptomic atlas reveals genes expressed in bone development are also expressed in the aortic valve

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In calcific aortic valve disease (CAVD), the upregulation of calcification promoters and the loss of mineralisation inhibitors contribute to abnormal mineralisation within the aortic valve. In this study, a sheep cardiovascular transcriptomic atlas was generated using RNA-seq with the aim of further understanding normal gene expression patterns in the context of the known physiology of healthy mammalian tissues.

Tissues included the mitral, aortic and tricuspid valves, as well as left and right auricles and ventricles from 2-year-old sheep. Detailed functional clustering of the sheep transcriptome was performed, where transcripts were grouped according to their expression pattern. This analysis, using the Miru (Kajeka) network analysis tool, was based on a gene-to-gene comparison of the expression patterns across analysed samples, using a Pearson correlation matrix (correlation value R≥0.99). Expressed genes in clusters were grouped together according to similarity of expression patterns across the tissues samples.

A sample-to-sample analysis showed that gene expression in the cardiac valves were distinct from that in the myocardium, confirming gene expression profiles reflect tissue type. Subsequently, a gene-to-gene analysis examined 11,942 genes, with >1.5 million connections, and 824 clusters of co-expressed genes in this dataset. Notably, one cluster that was highly expressed in the valves, particularly the aortic valve, contained genes expressed in normal bone development. Gene ontology enrichment analysis for this cluster returned genes involved in cell migration, glycosaminoglycan metabolic and collagen catabolic processes (Benjamini corrected p-values<0.001). Furthermore, 23/280 genes in this cluster were enriched for ossification and osteoblast differentiation (p-values<0.001), including bone morphogenetic protein-4 (BMP4),

fibrillin-1 (FBN1), collagen type VI alpha 1 (COL6A1), SRY-box 8 (SOX8), and bone gamma-carboxyglutamate protein (BGLAP).

This dataset provides a highly valuable resource for understanding gene expression in the healthy mammalian cardiovascular system. This approach enables us to capture and visualise the complexity of the relationships between different genes that maintain specialised cardiovascular functions. Our preliminary results indicate that genes commonly associated with bone development are expressed in the aortic valve, which may predispose to ectopic calcification in a permissive environment.

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P24

A novel role for extracellular signal-regulated kinase (ERK 2) in the control of matricellular protein secretion in osteosarcoma cells

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Objectives: Matrix deposition is tightly controlled during development but its dysregulation is a hallmark of cancer. In bone, cues from the extracellular environment such as signals from growth factors and mechanostimulation determine directionality of matrix deposition, which is mediated by the intracellular protein trafficking pathway. To analyse how these extracellular signals modulate intracellular protein trafficking we investigated a key downstream signalling pathway, the ERK-Mitogen-activated protein kinase (MAPK) pathway.

Methods: Osteosarcoma cells expressing a GFP chimera of the matricellular protein SPARC were imaged by total internal reflection microscopy before and after inhibition of the MAPK pathway by either a specific inhibitor of ERK 1 and 2 activation (U0126) or siRNA directed against ERK 1 or ERK 2. Trajectories of SPARC-GFP containing vesicles were analysed by calculating mean square displacement curves. Localisation of matricellular proteins was detected with immunofluorescence using specific antibodies. Protein secretion was measured with ^{35}S pulse chase experiments.

Results: Mean square displacement curves of SPARC-GFP containing vesicles show that inhibition of ERK1/2 activation impedes trafficking (38% reduction in displacement compared to control; p=0.02). Using siRNA directed against ERK 1 or ERK 2, we demonstrate that ERK 2 is important in the forward trafficking of endogenous matricellular proteins SPARC and osteopontin. Only downregulation of ERK 2 results in an altered intracellular protein localisation displayed as an accumulation of SPARC and osteopontin in the perinuclear region. This correlates with a lower level of total protein secretion as measured by ^{35}S pulse-chase experiments (32.7% reduction; p=0.0097).

In conclusion: our findings highlight the non-redundant role that ERK 2 plays in intracellular trafficking of matricellular proteins and support the hypothesis that growth factor signalling in bone regulates not only gene transcription but also the secretion of key proteins important for bone morphogenesis that are dysregulated in cancer.

P25

Characterizing the epidemiology of hypophosphatasia in a population in the United Kingdom

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Objective: To determine the prevalence and characterize the manifestations of hypophosphatasia (HPP) in the UK using an algorithm adapted to UK electronic health records.

Methods: We searched the Clinical Practice Research Datalink for patients diagnosed with HPP or phosphorus/phosphatase disorder by Read or ICD-10 code, respectively, and selected those with ≥1 clinical manifestation of HPP or all low alkaline phosphatase (ALP) results. Excluded patients had a diagnosis of hypophosphataemia or vitamin D deficiency, received bisphosphonate therapy, or had premature birth with only respiratory manifestations. Point prevalence was calculated based on data available as of 30 June, 2013.

Results: We identified 107 candidate patients with HPP; the estimated point prevalence was 0.96 (95%CI 0.71-1.29) per 100,000 population. Sixteen patients (15%) were identified by low ALP only. Of 91 identified by clinical manifestation, 59 (65%) had no ALP tests, 3 (3%) had all low ALP results, and 29 (32%) had normal or mixed low/normal results. The following comorbidities were recorded by age group at diagnosis: *0-6 months (n=36 [34%]):* respiratory failure (30 [83%]), seizure (15 [42%]), rickets (8 [22%]), nephrocalcinosis (5 [14%]), craniosynostosis (1 [3%]); *7 months-5 years (n=13 [12%]):* seizure (7 [54%]), respiratory failure (4 [31%]), rickets (2 [15%]), craniosynostosis (2 [15%]), nontraumatic fracture (1 [8%]); *6-17 years (n=9 [8%]):* rickets (3 [33%]); seizure (1 [11%]), dental anomaly (1 [11%]); *18-59 years (n=33 [31%]):* multiple/nontraumatic fractures (15 [45%]), rickets (3 [9%]), osteomalacia (3 [9%]), craniosynostosis (1 [3%]), nephrocalcinosis (1 [3%]); ≥60 years (n=16 [15%]): multiple/nontraumatic fractures (10 [63%]), chondrocalcinosis (2 [13%]).

Conclusions: The prevalence of HPP fell within the bounds of previous estimates. Potential data source limitations included misapplied clinical codes, underrecorded dental manifestations, and missing ALP results from specialists. Perinatal deaths in patients not registered with a general practitioner were not captured and may have been a source of underestimation.

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P26

Does arterial calcification require aerobic glycolysis?

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Objective: The process of arterial calcification shares many similarities to skeletal mineralisation, and involves the deposition of hydroxyapatite in the arteries. However, the cellular mechanisms responsible have yet to be fully elucidated. Accumulating evidence suggests that aerobic glycolysis (the Warburg effect), plays a critical role in meeting the demand for energy and biosynthetic precursors during proliferation and differentiation in numerous cell types. Therefore we addressed the hypothesis that vascular smooth muscle cell (VSMC) calcification requires aerobic glycolysis to produce energy and the necessary biosynthetic precursors.

Methods: Calcification of murine aortic VSMCs was induced by 3mM Pi for 7 days. Calcium deposition was determined using alizarin red staining and a modified o-cresolphthalein method. VSMCs were

cultured with the fluorescent glucose analogue 2-(N-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl)Amino)-2-Deoxyglucose (2-NBDG) to determine changes in glucose uptake. Gene expression was analysed by qRT-PCR.

Results: Calcium deposition was significantly increased in VSMCs cultured in 3mM Pi versus control conditions (124%, p<0.001). Calcified VSMCs also showed increased mRNA expression of *Runx2, Phospho1, Ocn* and *PiT-1* (P<0.001), recognised osteogenic markers of arterial calcification. Furthermore 3mM Pi treatment increased glucose uptake (98%, p<0.05) and Glut-1 mRNA expression (1.47 fold, p<0.001). Glycolysis converts glucose to pyruvate which is subsequently converted to either (i) acetyl-CoA by the pyruvate dehydrogenase complex (PDH) or (ii) lactate by lactate dehydrogenase (LDH). Notably, decreased VSMC calcification was observed in cells treated with sodium dicholoroacetate, an inducer of PDH activity (1mM; 40%; P<0.01) and citric acid, synthesised in the mitochondria from acetyl CoA (1mM; 72%, P<0.001). Treatment with the LDH inhibitor sodium oxamate (20mM) or sodium lactate (50 mM) to induce pyruvate production also inhibited VSMC calcification (68% and 53% respectively, P<0.05). Activation of the Wnt pathway - an established regulator of Warburg metabolism - using the selective GSK3 inhibitor CHIR99021 (1nM) significantly increased VSMC calcification (417%, p<0.001). However, co-treatment with sodium oxamate (20mM) significantly blunted the pro-calcification effect of CHIR99021 (69%, p<0.01).

Conclusion: Together these data suggest that arterial calcification requires glucose metabolism through a mechanism involving Wnt signalling. Interruption of the glycolysis pathway may therefore represent a novel therapeutic target for clinical intervention.

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P27

Bioenergetic characterisation of osteoblastic and chondrocytic cells under basal and stressed conditions through extracellular flux analysis

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Objectives: The function of osteoblasts, from proliferation and early differentiation, through bone formation and mineralisation to final differentiation to osteocytes, requires energy from two major energy-producing pathways of the cell; mitochondrial respiration (MR) and glycolysis. This study measured 'real-time' MR and glycolysis in osteoblastic and chondrocytic cells with specific focus on whether osteoblastic differentiation was associated with a shift in energy metabolism.

Methods: We used primary human osteoblast (HOBS) and three osteosarcoma cell lines representing different stages of osteoblast differentiation, MG-63, TE85 & Saos-2, and the C20 chondrocyte cell line. MR and glycolysis were measured by extracellular flux (ECF) analysis using an Agilent Seahorse XFp, which analyses the composition of the culture medium during the assay. MR and glycolysis were indicated by oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) respectively. Measurements were taken under basal then stressed conditions following combined addition of oligomycin and FCCP (carbonyl cyanide-4-(trifluoromethoxy) phenylhydrazone) which simultaneously inhibit MR and glycolysis, stimulating a compensatory increase in MR and glycolysis to meet energy demands.

Results: Readings were the average of three replicates, normalised to total cell content (DNA) to give relative OCR and ECAR intensity values. ECF analysis revealed key differences between cell types for OCR (Saos2: 0.07(SD=0.0044), TE85: 0.053(SD=0.011), MG-63: 0.059(SD=0.012), C20: 0.03(SD=0.0048), HOBS: 0.12(SD=0.034)) and for ECAR (Saos2: 0.02(SD=0.0021), TE85: 0.04(SD=0.009), MG-63: 0.03(SD=0.002), C20: 0.053(SD=0.0055), HOBS: 0.03(SD=0.012)). C20 chondrocyte cells were the most glycolytic under basal conditions, consistent with their expected phenotype *in vivo*. HOBS had the highest basal rate of MR and the greatest compensatory reaction to stressors in both measures. Surprisingly Saos-2, the most osteocytic of the cell lines, showed the lowest ECAR rates.

Conclusion: ECF analysis indicated key metabolic differences between the cells under study in basal conditions and in response to increased energy demands. Dependence of the chondrocytic cells on glycolysis is consistent with the low oxygen tension of their tissue environment *in vivo*. In contrast, there was no evidence of a switch from MR to glycolysis in Saos-2, which express an osteocytic phenotype.

P28

Do blood lipid levels influence bone mineral density? Findings from a Mendelian randomization study

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Objectives: Treatment with statins, which are widely used as cholesterol-lowering agents, is associated with increased bone mineral density (BMD) and reduced fracture risk. Previous laboratory studies suggest that direct effects of statins on osteoblasts and/or osteoclasts underlie this relationship. However, few studies have investigated the alternative possibility that beneficial effects of statin use on bone are mediated by lowering cholesterol levels. In order to investigate these relationships, we performed a two-sample Mendelian randomization (MR) study.

Methods: We utilized 184 single nucleotide polymorphisms (SNPs) robustly associated with plasma lipid levels and 239 SNPs associated with heel BMD derived from quantitative ultrasound in 142,487 individuals from the UK Biobank. We performed univariate MR analyses on LDL-cholesterol (LDL-C), HDL-C and triglyceride levels, as well as multivariable MR to account for genetic pleiotropy among the different lipid fractions. To test whether the effect of statins on BMD is mediated by lowering lipid levels, MR was repeated using a *HMGCR* SNP, rs12916, which mimics effects of statins on LDL-C. We also performed bi-directional MR to examine the reverse causality between BMD and blood lipids. Analyses were conducted using genetic association summary statistics from MR-Base (www.mrbase.org).

Results: Univariate MR using SNPs associated with LDL-C, HDL-C or triglyceride levels provided evidence for a causal effect of LDL-C on BMD (beta=-0.077, $P=2x10^{-5}$; SD change in BMD per SD change in LDL-C). Multivariable analysis suggested that the effect of LDL-C on BMD was independent of HDL-C and triglycerides. MR using the *HMGCR* SNP, rs12916, was consistent with a causal effect of LDL-C on BMD (beta=-0.116, P=0.013) as were MR analyses excluding this SNP(beta= -0.075, $P=4x10^{-5}$). Bidirectional MR analyses

provided no evidence of reverse causality for an effect of BMD on any blood lipids.

Conclusions: Our results suggest lower LDL-C is causally related to increased BMD. This suggests the effect of statins on BMD is at least partly due to their LDL-C lowering effect. Further studies are justified to explore the mechanisms by which lower LDL-C improves BMD, and to examine their potential role in treating osteoporosis.

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Cordycepin reduces established bone changes and pain in two animal models of osteoarthritis

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Osteoarthritis (OA) is a common cause of joint pain and disability in the ageing population. OA involves all joint tissues, however, subchondral bone (SB) remodelling together with cartilage damage are considered to be hallmarks of OA. Treatments targeting subchondral bone changes have a huge potential in controlling OA.

In-vitro and in-vivo studies support potential benefits of cordycepin (3'deoxyadenosine) in preventing bone loss through inhibition of osteoclast generation/differentiation and having an osteoprotective effect in pathologies such as osteoporosis.

The aim of this study was to determine whether cordycepin treatment alters osteoarthritic pain and joint pathology, and to decipher the mechanisms of action by which cordycepin exerts any potential beneficial actions.

OA was induced in rats by injection of mono-sodium iodoacetate (MIA; 1 mg/50 µl) on day 0 and in mice by medial meniscus displacement (DMM). Cordycepin was administered orally (8 mg/kg or vehicle) every other day for 2 weeks (MIA: day 14 to day 28. DMM: week 14 to week 16). Pain behaviour was measured as hind-limb weight-bearing asymmetry. Joint changes (cartilage and bone damage [osteophytes, integrity of the osteochondral junction [OCJ], SB remodelling]) were quantified using histology and immunohistochemistry (tartrate-resistant acid phosphatase [TRAP] positive osteoclasts) techniques.

The MIA rat model and the DMM mice model of OA exhibited significant pain behaviour, cartilage damage, osteophyte formation and SB changes, compared with controls. A two week therapeutic treatment with cordycepin reduced the pain behaviour in both of the models of OA. In the MIA model, cordycepin reduced the number of channels crossing the OCJ and TRAP positive osteoclasts in the SB, but had no effect on numbers of osteophytes or cartilage damage. In the DMM model, cordycepin treatment reduced cartilage damage, abnormal bone changes and osteophyte growth.

Our data from two animal models, a fast progressing chemical model (MIA) and slow progressing surgical model (DMM) show that the analgesic effects of orally administered cordycepin are associated with bone remodelling. Further studies will investigate the detailed mechanisms of action with which cordycepin exerts its beneficial effects and could therefore prove to be a novel drug for treating OA.

Supporter: The work was supported by a grant from Arthritis Research UK.

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Mechanical stimuli counteract the deleterious effects of hyperglycemia on the bone quality and fracture healing in diabetic rats

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In order to assess the effects of mechanical stimuli on the bone quality and fracture healing in diabetic and non-diabetic rats, 112 female Wistar rats (200±10 g) were assigned to four groups: (1) SHAM, (2) sham with vibration therapy (SHAM+VT), (3) diabetes mellitus (DM), (4) DM+VT. Diabetes was induced with a single intravenous injection of streptozotocin[1]. Thirty days after diabetes induction, animals underwent closed bone fracture at the right mid-femur, followed by surgical stabilization of bone fragments[2]. Three days after bone fracture, rats began VT. On days 14 and 28 post-fracture (representing two distinct phases of normal bone healing: soft and hard bone callus), the rats were euthanized, blood was collected for serum bone marker analysis, and both femurs were collected for bone densitometry, micro-computed tomography and histological analysis. Diabetes led to a dramatic impairment of both bone quality and fracture healing in both end-points assessment (with a time-dependent feature). In intact bone, diabetes decreased bone mineral density (BMD) by 45% and caused detrimental changes in bone microarchitecture (reduced BV by 90%, BV/TV by 87%, TbN by 85% and Conn.D by 77%). In bone healing, diabetes caused a delay in cell proliferation; reductions of 17% in BMD, 81% in callus volume, 69% in mineralization, 93% in IGF-1; and a 1385% increase in CTX-I levels. VT was effective at improving both bone quality and fracture healing. In intact bone, VT increased BMD in 20% and ameliorated trabecular microarchitecture (augmented BV by 494%, BV/TV by 386%, TbN by 394% and Conn.D by 233). VT accelerated osteogenic and chondrogenic cell proliferation at the fracture callus; increased circulating IGF-1 by 839%, RANK-L by 19%, callus volume by 52%, bone mineral content by 90%, and callus area by 72%; and was associated with a 19% reduction in circulating RANK-L. In conclusion, diabetes had detrimental effects on both non-fractured bone quality and fracture healing. Vibration therapy was very effective at counteracting the significant disruption in bone metabolism, mass and microarchitecture on both non-fractured and fractured femurs of diabetic rats.

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High magnitude vertical impacts are positively related to hip strength in master athletes

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Objectives: Little is known about how high impact physical activity (PA) relates to bone in older adults. This study examined cross-sectional associations between PA vertical impact magnitude and hip strength in older adults training for sprint and endurance running events.

Methods: Study participants were UK-based master athletes who had competed in sprint or long distance running events in the past 12 months at a regional level at time of recruitment. Vertical axis peaks from seven-day accelerometer recordings were used to classify PA as low (0.5<g<1.0 g), medium (1<g<3.5 g) or high (≥3.5 g) impact. Total hip bone mineral density (BMD) and cross-sectional moment of inertia (CSMI) were measured by DXA. β-CTX was extracted from fasting blood samples. Associations were examined using linear regression models adjusted for age, sex, athletic event, height and total body fat and lean mass. Results were presented as standard deviation changes in outcomes (plus 95% confidence intervals) per doubling in number of impacts.

Results: 166 master athletes with a mean age of 69 (range=59-85) years were included in analyses (27.7% female, 20.5% sprinters). Hip BMD and CSMI were greater in sprinters compared with endurance runners (+0.11 g/cm^2 for hip BMD), and in males compared with females (+0.15 g/cm^2 for hip BMD). Overall, high impacts were associated with higher hip BMD [0.038 (0.001, 0.074)] and hip CSMI [0.031 (0.004, 0.057)]. Subgroup analyses showed high impacts were positively associated with hip BMD in endurance runners [0.049 (0.010, 0.087)] but not sprinters [-0.019 (-0.132, 0.094)] and in males [0.053 (0.006, 0.100)] but not females [-0.026 (-0.087, 0.034)]. High impacts were associated with higher β-CTX in females [0.031 (0.009, 0.054)] whereas an equivalent association was not seen in males [-0.032 (-0.117, 0.054)]. Low and medium vertical impacts were unrelated to outcomes.

Conclusion: High but not medium or low magnitude vertical impacts were associated with higher hip BMD and CSMI in master athletes however, associations varied according to athletic event and sex. Weaker associations between high impacts and hip BMD in sprinters and females may reflect their higher pre-existing BMD and bone turnover respectively.

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The ontogeny of human vertebral bone microstructure: implications for mechanical performance

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Gestation, infancy and childhood are periods of rapid bone growth (increase in size) and development (change in shape), and studies of these changes suggest that human trabecular growth and development is dynamic and sequential. Much less is understood about how such changes in 3D architecture contribute to mechanical performance. The aim of this study was to evaluate structural differences in juvenile vertebrae and develop a methodology for their non-destructive mechanical testing, using finite element modelling (FEM). A series of lumbar vertebrae taken from 19th museum collections of skeletons of documented age at death, between 6months gestation and 5years postnatal, were visualised using microcomputed tomography. Specimens underwent stereological and graphical analysis, employing Bone J (v.1.4.1), including calculation of the bone volume to total volume ratio, total volume, trabecular thickness, trabecular spacing, and anisotropy. Image stacks of the vertebrae stripped of their cortical shells were generated and reduced to a voxel thick line between trabecular junctions/nodes. Nodes and branches were identified, and reconstructed into weighted directed graphs using Python (v.2.7.12), Numpy (v.1.11.1) and the parallel-processing library Multiprocessing. Segmented images were imported into an FE meshing tool (Scan IP/FE Simpleware Ltd) and a model of each vertebra was generated using tetrahedral linear elements. These were imported into FE software (Abaqus CAE v.6.9-1); a distributed compressive load was applied in three positions; the models were solved and the stiffness determined for each specimen and each position to investigate the material and structural evolution between the developmental stages, and the variation of properties as a function of loading position. The variation in trabecular architecture with age suggests a sequential pattern of growth and remodelling, and a trend towards homogenisation of 3D structure across the cancellous bone of the vertebral body. However, within the trabecular architecture the number of branch connections per node remains relatively stable. The findings from the FEM indicate that these 3D structures underpin different mechanical responses both within specimen models depending on the position of the applied load, and between specimens, with young fetal and infant vertebrae exhibiting high stiffness in the medial loading position.

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ETS2 Repressor Factor (ERF) - associated craniosynostosis: molecular mechanisms and therapeutic approaches

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Objectives: Haploinsufficiency of the transcriptional repressor ERF causes craniosynostosis - premature ossification of cranial sutures - in humans and mice. Our aim is to elucidate the mechanisms by which ERF affects cranial suture fate, as well as to identify pharmacological substances with potential therapeutic application in craniosynostosis.

Methods: We utilized the *Erf$^{loxP/-}$* craniosynostosis mice and generated *Nestin-cre/+;Erf$^{loxP/loxP}$* mice that lack expression of *Erf* in mesenchymal and neuronal progenitors, to establish cultures of primary suture- derived mesenchymal cells and compare them to cells derived from their non- affected siblings. We tested selective XPO1 and ERK1/2 inhibitors that can increase nuclear Erf accumulation for their ability to alleviate the cranial phenotype of *Erf$^{loxP/-}$* mice.

Results: Cells isolated from craniosynostotic mice exhibit increased early- stage osteogenic differentiation, but diminished late- stage mineralization. Culture of CD105$^+$ suture osteoprogenitor cells under osteogenic conditions showed the same abnormal pattern suggesting a cell- autonomous effect. Interestingly, suture cells isolated from craniosynostotic mice show increased potential for chondrogenic and adipogenic differentiation, indicating an increased proportion of mesenchymal stem cells as a result of decreased Erf levels. The administration scheme of the selective inhibitors that we followed in our studies resulted in the improvement of skull morphometry, implying that continuous ERF presence is required for normal suture development.

Conclusion: Our so far data suggest that Erf has a dual role: it controls matrix calcification during osteoblast differentiation in intramembraneous ossification, but it may also act upstream, in the commitment of mesenchymal stem cells towards their lineages. Ongoing experiments aim to find the specific targets of this factor that are particularly involved in craniosynostosis, and to make

alterations in the method and timing of drug administration for the complete restoration of the normal phenotype.

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Generation of the HGD mouse model of alkaptonuria

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The current AKU mouse model used in research (referred to as AKU) was generated by chemical mutagenesis via injection of the mutagen N-ethyl-N-nitrosourea (ENU). Due to the nature of chemical mutagenesis, this model potentially has thousands of uncharacterized mutations that could affect the disease phenotype. We wanted to create a specific HGD null mouse with the advantage of being a conditional deletion in C57BL6 background.

Embryonic stem (ES) cells with disrupted HGD gene function were generated by the Knock-Out Mouse Project (KOMP) and obtained from their repository (www.komp.org). This knock out first plasmid contained the insertion of an IRES:lacZ trapping cassette and a promoter-driven neo cassette into the fifth intron of the HGD gene while the sixth exon was flanked by LoxP sequences (HGD tm1a)[1]. Flp recombinase reverts the mutation back to wildtype with a floxed critical exon (HGD tm1c). The model then becomes conditional though Cre recombinase which removes the floxed exon resulting in a mutant transcript (HGD tm1d). The other advantage of this system is that ES cells are in C57BL6 background.

Homozygote tm1a mice showed black urine-stained cage bedding demonstrating the first observed AKU symptoms (due to the oxidation of HGA). The homozygotes have hugely elevated urinary HGA (32,082-106,020 µmol/L), measured by mass spectrometry, compared with both the heterozygous (2-45 µmol/L) and WT (20 µmol/L) mice. There is no evidence of haploinsufficiency.

To demonstrate where the HGD gene is expressed, we used the β-galactosidase transgene that is knocked in to disrupt the gene. Surprisingly no staining was observed using LacZ suggesting that the transgene was not active. We show a comparison of the new HGD null and AKU mouse and explore the conditional deletion of HGD in liver and the level of required HGD to rescue the phenotype.

We present a novel conditional HGD null mouse that is specific to the HGD gene as a model of alkaptonuria.

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P35

Evaluation of the Utah algorithm for identifying hypophosphatasia to estimate prevalence in the United Kingdom

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Objective: Determining the prevalence of hypophosphatasia (HPP) presents substantial challenges due to lack of recognition of the diagnosis; estimated perinatal/infantile HPP prevalence is 1:300,000 births. We retrospectively applied the Utah HPP algorithm to estimate HPP prevalence in the UK.

Methods: The Utah algorithm selects patients with (1) ≥1 record with the ICD-9 code for phosphate metabolism disorders or ≥2 low age-/sex-adjusted alkaline phosphatase (ALP) results; (2) ≥1 clinical HPP manifestation; and (3) no hypothyroidism or bisphosphonate therapy. We accessed routine UK primary care and linked hospital data and adapted the algorithm's diagnostic component to select for Read and ICD-10 codes to estimate prevalence at 30 June, 2013.

Results: Of 14.4 million patients, 306 had Read codes for HPP, 1589 had ICD-10 codes for phosphate/phosphatase disorders, and 10,660 had low ALP. Of the selected patients, 590 had ≥1 manifestation; of these, 259 patients had all low ALP results; and of these, 256 had no HPP diagnostic record. Estimated prevalence was 2.57 (95%CI 2.15-3.10) per 100,000 population. Three patients (1%) presented at age 7 months-5 years, 22 (8%) at 6-17 years, 186 (72%) at 18-59 years, and 48 (19%) at ≥60 years. Whether ALP reference ranges were age-/sex-adjusted or all ALP histories were complete (only tests commissioned in primary care were available) was unknown. No ALP measurements were recorded for 233/590 (39%) patients meeting all non-ALP selection criteria.

Conclusions: The prevalence of HPP was higher than expected, with little overlap between algorithm-identified and physician-diagnosed patients. Arbitrarily low ALP measurements may have precipitated false positives where combined with non-HPP-specific clinical manifestations, and false negatives may have occurred where ALP history was unavailable or reference ranges were not appropriately adjusted. Thus, the Utah algorithm with its reliance on the low ALP criterion is unsuitable for application in UK electronic healthcare records; an alternative means of identifying HPP is needed.

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Bone marrow adipose tissue: a novel regulator of metabolic and skeletal health?

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Bone marrow adipose tissue (MAT) accounts for 70% of bone marrow volume and over 10% of total adipose mass in lean, healthy humans. MAT further increases in diverse clinical conditions, including ageing, osteoporosis, cancer therapy and, strikingly, during caloric restriction. However, study of MAT has been relatively

limited, and therefore the formation and function of MAT remains poorly understood. We previously revealed that, like white adipose tissue, MAT is an endocrine organ that can exert systemic effects. My lab is now building on this finding by investigating the causes and consequences of MAT formation, in particular during caloric restriction, with the goal of determining how MAT impacts metabolic and skeletal health.

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Hepcidin modulates *runx2a* gene expression to regulate biomineralization of bone in zebrafish

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Background: Iron overload, as a risk factor for osteoporosis, can result in the up-regulation of *Hepcidin*, and *Hepcidin* knockout mice display defects in their bone microarchitecture. However, the molecular and genetic mechanisms underlying *Hepcidin* deficiency-derived bone loss remain unclear.

Method: We show that knockdown of *hepcidin* in zebrafishusing morpholinos leads to iron overload. We also used CRISPR-Cas9, a versatile genome-editing tool, to generate a zebrafish *hepcidin* mutant.

Results: Iron overload and a mineralization delay were observed in osteoblast cells in *hepcidin-/-* zebrafish larvae, and these defects could be partially restored with a microinjection of *hepcidin* mRNA. Quantitative real-time PCR analyses revealed the down-regulation of the osteoblast-specific genes *alp, runx2a, runx2b,* and *sp7* in homozygous *hepcidin* mutant zebrafish. Luciferase reporter assays further showed that bone morphogenetic protein 2a(Bmp2a) enhanced the expression of *runx2a*, while iron overload repressed its expression through *bmp2a* (independent of *hjv*). High-throughput transcriptome analysis of *hepcidin-/-* zebrafish revealed multiple pathways involved in osteoblast metabolism.

Conclusion: These findings show that iron overload derived from *hepcidin* deficiency represses bone formation, possibly through the BMP pathway, and affects *runx2* in zebrafish.

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Everyday physical activities in older people with compression vertebral fractures (VFs): a systematic review and meta-analysis

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Objectives: Older people with VF have worse physical Health Related Quality of Life (HRQoL) than older people without VF. However, it's not clear exactly what aspect of physical activities (PAs) is impaired in people with osteoporotic VF. Further understanding of the effect of fracture on some activities would allow the development of appropriate interventions to improve quality of life

Methods: A comprehensive search was undertaken using the databases of PubMed, Embase, Medline, Web of Science, and the "grey" literature from 1950 to the end of July 2016. Standardise search terms for VF and PAs were used. Two categorise of PA were included (1) bending activities (including bending, Getting in or out car, Putting on socks or stockings, Lifting a 5kg object from the floor) (2) Ambulatory activities (include walking, Use of a walking aid, using stair). Strict inclusion and exclusion criteria were used and only studies that matched VF cases and controls for age and gender were included. For the meta-analysis, pooled OR and 95% confidence interval (CI) were calculated using a random-effects model.

Results: 12 studies in total were identified which had investigated the associations between prevalent VF and the selected PAs, and expressed these as ORs (or RR). For bending activities, there was an association between VF and bending problems for both women (*seven studies*) and men (*two studies*) (1.49,(1.22,1.83), P<0.001) and (1.87,(1.14,3.07), P= 0.013) respectively (OR, 95%CI). Lifting 5Kg object from the floor was the most affected activity in women (four studies) (1.95,(1.41,2.68), P<0.001). For ambulatory, there was an association between VF and reduced ambulatory activities for women (*9 studies*) but not for men (*four studies*) (1.27,(1.14,1.42), P<0.001) and (1.09,(0.91,1.32), P=0.346) respectively. Climbing 10 steps (2.37,(1.68,3.36), P<0.001) was the most affected ambulatory activity in women (*three studies*).

Conclusion: Studies constantly show women with VF have reduced bending and ambulatory activities, while much less research has been carried out in men. Our study will help physiotherapists to develop more specific programs to improve HRQoL in women with VF.

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Prevalence of obesity, osteopenia and osteoporosis in adults with cerebral palsy

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Children with cerebral palsy (CP) have an underdeveloped musculoskeletal system and low levels of physical activity compared to typically developing children. Children with CP experience declines of mobility as they age into their adult years, exposing adults with CP to an accelerated development of age-related chronic diseases. However, little is known about the prevalence of obesity and osteopenia/osteoporosis in adults with CP across the lifespan.

A clinic-based sample of adults with CP (n=1655; age 37.4±15.1 years; 48% women) was examined. Prevalence of obesity and osteopenia/osteoporosis was evaluated. Multiple logistics regression models were developed (n=1272) to determine if sex, age, functional mobility and body mass index (BMI) are significant predictors of osteopenia/osteoporosis in adults with CP. Functional mobility was determined using the Gross Motor Function Classification System (GMFCS), where those classified as I-III have more functional mobility than those classified as IV-V.

Based on BMI, 39.4% of adults were underweight, 18.4% were normal weight, 21.2% were overweight and 21% were obese. The prevalence of osteopenia/osteoporosis was 39.3%. In the fully adjusted model, there were no differences in the odds of osteopenia/osteoporosis based for sex or functional mobility status (compared to GMFCS I-III, OR: 0.98, 95 % CI: 0.78-1.24). However, age was independently and positively associated with osteopenia/osteoporosis prevalence (reference: 18-25.9 years; 26-39.9 years: OR: 1.77, 95 % CI: 1.33-2.37; 40-59.9 years: OR: 1.71, 95 % CI: 1.25-2.34; ≥60 years: OR: 1.83, 95 % CI: 1.16-2.88), and overweight/obesity status was negatively associated

with osteopenia/osteoporosis prevalence (reference: underweight; overweight: OR: 0.70, 95 % CI: 0.48-1.00; obese: OR: 0.69, 95 % CI: 0.47-1.01).

This is the largest study to date on chronic disease prevalence in the CP population. The prevalence of osteopenia/osteoporosis in adults with CP increases substantially by about the 3rd decade of life, which is maintained with increasing age. Overweight and obesity status are skeletally protective and preserve bone mineral in adults with CP. Future studies are needed to determine intervention strategies to ensure preservation of lean mass in this population.

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A method to analyse the development of osteolytic lesions in mice using *in vivo* micro-computed tomography (µCT) and rigid image registration

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Osteolytic lesions are a key feature of diseases such as multiple myeloma and metastatic breast and prostate cancer; and the development of µCT has allowed these lesions to be visualised and measured in murine models of cancer. More recently, the development of *in vivo* µCT has made it possible to detect and map the progress of bone lesions over time. However, currently there is no established method to analyse their growth. Here, using a myeloma xenograft model, we have developed a method to longitudinally image and analyse bone lesions. NOD/SCID-γ mice were injected intravenously with $1x10^6$ human myeloma U266 cells, where bone lesions are known to increase over time. After 6 and 9 weeks post-tumour cell injection the right tibia of each tumour-bearing mouse was imaged using an *in vivo* µCT scanner. The resulting longitudinal datasets (N=6) were then analysed for bone lesion growth over time using volume rendering software. Images were initially manual orientated, before we next examined the suitability of using rigid image registration to improve the robustness of the method. We found that image registration improved reproducibility, with analysis of repeated datasets resulting in 1.3% variation compared to 21.1% when repeatedly analysing the same manually orientated dataset. Furthermore, we found that image registration led to better accuracy, with bone lesion area of unregistered datasets varying from that of registered datasets by an average of 10.1%, and that image registration had the potential to improve trabecular bone measurement accuracy. In summary, in vivo µCT offers a novel way of monitoring the development of bone disease over time in *in vivo* murine models of cancer, and importantly, image registration improves the robustness of analysing bone lesion growth.

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Osteitis condensans ilii: Differential diagnosis and management

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Osteitis condensans ilii refers to an increase in bone density located on the inferomedial aspect of the ilium adjacent to the sacroiliac joint. It is often bilateral, symmetric, and triangular. It is a rare condition of benign cause of axial low back pain. Typically affects females following pregnancy, but males and nulliparous females have also been reported.

Clinical problem: 29 years old woman presents with lower back pain and polyarthralgia without a history of trauma or injuries. The condition affects her daily life activities and disturbs her night sleep. Finds it particularly difficult to lie flat and get in and out of bed. No other comorbidites recorded.

Clinically: Paraspinal and midline tenderness at L4/L5 levels, Good spinal flexion, neurovascular intact. Hip examination unremarkable. Distraction, Compression FABER and Gaenslen's test for sacroiliac joints were positive.

Radiology findings demonstrate well defined sclerotic area, adjacent to Left SIJ only on the iliac side. The SIJ is preserved without any joint erosions. Further MRI scans of LS spine and pelvis were performed to rule out other reasons for LBP.

Laboratory studies have shown: Vit D deficiency, Hyperthyroidism, Normal CRP and ESR and Rh factor.

Further testing for HLA-B27antigens is performed.

Conservative treatment modalities like physiotherapy and non-steroidal anti-inflammatory medication was employed.

Results: Conservative measures taken have led to satisfactory symptoms control.

Discussion: OCI is a rare cause of lower back pain. XR and MRI isneeded to diagnose and exclude other reasons for lower back pain. Further laboratory tests are also required to distinguish this idiopathic condition from ankylosing spondylitis, seronegative spondyloarthropathy, metastatic disease or sacroiliitis.

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P46

The musculoskeletal benefits of gait speed maintenance in older men and women

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Objectives: There are few data on the relationships between measures of mobility, such as gait speed, and longitudinal changes in musculoskeletal parameters. We investigated these associations using a well phenotyped cohort of older men and women.

Methods: We studied 172 men and 156 women from the

Hertfordshire Cohort Study, each of which underwent peripheral quantitative computed tomography (pQCT) of the tibia (38%) in 2004-5 and then again in 2011-12. Gait speed was assessed for all of these at baseline (3-metre walk) and for 164 men and 147 women at follow-up (8-foot walk). Percentage change per year was calculated for gait speed, muscle cross-sectional area (mCSA), fat cross-sectional area (fCSA) and diaphyseal bone parameters (total area (Tt.Ar), cortical area (Ct.Ar), cortical density (Ct.BMD), and polar stress strain index (SSIp)). Relationships between gait speed and pQCT parameters were assessed using linear regression.

Results: The mean age of men and women at baseline was 68.9 and 69.3 years respectively. Mean (SD) follow up time was 7.17 (0.39) years. Mean (SD) baseline gait speed was higher in men than women at 0.94 (0.16) and 0.89 (0.16) m/s respectively (p=0.01). Rate of decline did not differ significantly by sex. A greater baseline gait speed was associated with a slower decline in diaphyseal Ct.Ar and SSIp in men and Tt.Ar in women. In men, rate of loss of gait speed was positively associated with rate of loss of Ct.Ar. In both men and women, a slower decline in gait speed was associated with a slower increase in fCSA and slower decline in mCSA although the latter association only reached statistical significance in women. All relationships were maintained after adjustment for the corresponding baseline bone, fat or muscle parameter (p<0.05).

Conclusion: Maintenance of gait speed is associated with a slower increase in subcutaneous fat in the lower limb. In men, the rate of loss of Ct.Ar is associated with both baseline and rate of loss of gait speed. This suggests that interventions to optimise and maintain mobility may help to ameliorate the age-related deterioration in bone health.

P47

A novel method for identifying radiographic baseline risk of osteoarthritis using an anisotropy-based texture analysis algorithm: data from the Osteoarthritis Initiative

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Background: Recent developments in imaging techniques showed that Osteoarthrtitis (OA) is not just a joint disease but also involves progressive changes in the subchondral/subarticular area of the tibia. On top of the accepted method of measuring joint space width (JSW), assessments of the trabecular bone structure (TBS) in selected regions of interest (ROI) using radiographs may be offering an alternative method for quantifying the risk and progression of OA.

Objectives: JSW has limited capabilities in regard to early identification of OA. The objective of this abstract is to evaluate the TBS as an area for early identification of OA risk, applying texture anisotropy algorithms and subsequently comparing the results to JSW measurements.

Study Design & Methods: This study was performed using data from the Osteoarthritis Initiative. The image data was restricted to female, Caucasian, right knee exams recorded with the same modality. Furthermore we selected exams which had KL grade of 0 at the baseline exam and a deteriorating KL ≥2 at 96 month follow up. 22 cases fulfilled these criteria and we selected 22 matching controls with no signs of OA at 96 month follow up. The selected ROI for the analysis of the radiographic texture consisted of 6 ROIs. For each individual ROI, the degree of texture anisotropy was calculated and compared between case/control. In addition, JSW was calculated in both groups using a proprietary software-based method.

Results: Whereas the JSW measurements did not yield any significant differences with respect to their mean values (Cohen's d= 0.139), the calculated texture parameters showed that differences in values between cases and controls can be found in two of the ROIs (ROI1&2) with Cohens'd values of 0.625 and 0.831, respectively. With respect to selected patient, the differences in anisotropy results were significant using these texture parameters.

Conclusions: Our results indicate that using texture parameters, an early identification of patients at risk for developing OA using X-rays can be achieved. This may offer an additional method for quantifying the risk of baseline OA.

P48

Horrendous pain surely not osteoarthritis (HPOA)

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Background: A 45-year-old man presented with widespread painful, swollen joints with associated high-grade fever, chronic cough and weight loss. He had a 15 pack year smoking history. On examination he had synovitis of the metacarpophalangeal joints, distal interphalangeal joints and ankle joints. He was also noted to have finger clubbing. Two months prior to this admission he was provisionally diagnosed with seronegative arthritis after presenting with joint pain to another hospital. Here radiographs of his hands and feet were reported as normal, and a chest radiograph showed a left-sided hilar mass.

Presenting problem: Repeat radiographs of his hands, feet and ankles showed novel, florid, periosteal elevation. A chest radiograph showed a large mass in the left mid-zone. A subsequent CT scan showed a 10x11x10 cm intrathoracic mass in the left upper lobe, invading into the mediastinum.

Clinical management: The history and radiographic changes led to a diagnosis of hypertrophic pulmonary osteoarthropathy (HPOA), a paraneoplastic phenomenon associated with his primary lung pathology, and not a primary arthritis as first thought. A CT-guided biopsy of his lung mass suggested a diagnosis of an inflammatory pseudotumour or a solitary fibrous tumour, although there were concerns the sample was not representative. For symptom relief he was given 30 mg pamidronate and 120 mg intramuscular methylprednisolone, which resulted in rapid improvement of his symptoms. He did not attend further follow up, but was admitted to a third hospital with a pericardial effusion. He deteriorated and died before further diagnostic clarification was possible.

Discussion: HPOA is a syndrome characterised by finger clubbing, periosteal reaction of distal extremities and synovial effusions. It is a rare condition usually occurring secondary to primary or metastatic lung carcinoma, or conditions associated with chronic lung or pleural inflammation.

This case emphasises the importance of a chest radiograph in the assessment of all patients with acute arthritis, particularly in previous smokers. HPOA can be rapidly progressive, demonstrated by the florid radiographic changes over two months. Finally, bisphosphonates used in combination with corticosteroids give rapid and effective pain relief, however ultimately the mainstay of treatment is definitive management of the primary pathology.

P49

Circulating levels of bone regulatory factors Dickoppf-1, Osteocalcin, Osteoprotegerin and Sclerostin are associated with whole-body bone mineral density in older men and women

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Purpose: To investigate the relationship between circulating levels of bone regulatory factors with whole-body bone mineral density (WBMD) during 'healthy' ageing.

Methods: We compared WBMD and body composition as well as fasting plasma concentrations of 1,25 dihydroxyvitamin D3 , Calcium (Ca), Dickoppf (DKK1), Fibroblastic growth factor (FGF-23), Osteocalcin (OC), Osteoprotegerin (OPG), Osteopontin (OPN), Parathyroid hormone (PTH), Sclerostin and Tumour necrosis factor (TNFα) between community dwelling older adults and a reference young adult population in 440 men and women (n=269 aged 69 to 81 years (52% female) and n=171 aged 18-30 years (53% female)).

Results: Compared with young, older adults had higher concentrations of DKK1, OPG, PTH, sclerostin and TNFα. Concentrations of Calcium and OC were lower in old compared with young, while 1,25 dihydroxyvitamin D3, FGF-23 and OPN did not differ significantly between young and older adults. Circulating DKK1, OC, OPG and sclerostin were individually positively associated with WBMD in the old (r-values ranging from .132 to .254). In young, DKK1 was positively associated with WBMD. DKK1, OC, OPG and sclerostin were each positively associated with WBMD in older adults, despite the average circulating DKK1, OPG and sclerostin being higher in old than young.

Conclusion: It is possible that healthy, older osteocytes release higher levels of DKK1, OPG and sclerostin into the circulation, but this tendency may be halted in osteoporotic bone where the trabeculae resorption is more advanced.

Key words: Whole body bone mineral density (WBMD), older, plasma, osteocytes.

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P50

The effect of age and puberty on proximal femur shape

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Objectives: While sex differences in hip geometry emerge during puberty, it is unclear whether other aspects of hip structure such as hip shape - linked to osteoarthritis later in life, show a similar relationship with puberty. Therefore, we examined relationships between puberty and hip shape in adolescents, and whether these associations differ to those related to chronological age.

Methods: Children from the Avon Longitudinal Study of Parents and Children, a UK population based cohort, were investigated. To quantify proximal femur shape, hip DXA images were analysed using Shape software (Aberdeen, UK) based on a 53-point Statistical Shape Model (SSM). To allow comparison between datasets, scores from an adult reference SSM (N=19379) were applied, and hip shape mode scores (HMs) for each image were generated. Differences in HM scores between age 13.8 and 17.8 were assessed by paired t-test. Associations between puberty stages (Tanner I+II, III and IV+V), assessed at an average age of 13.2 years, and HMs were examined by linear regression.

Results: Complete outcome and puberty data at age 13.8 years were available for 1924 children (882 boys and 1042 girls), and 1725 children (780 boys and 945 girls) had complete data for both time points. HM2 (variation in femoral neck width (FNW)) and HM3 (reflecting larger, less spherical femoral head) scores were inversely related to Tanner stage (p for trend <0.001) (males and females combined). HM2 score decreased between age 13.8 and 17.8 years with the mean difference in boys [-0.586(-0.626,-0.546) p<0.001] greater than in girls [-0.166(-0.197,-0.135) p<0.001]. In contrast, HM3 score increased with age and the mean difference in boys [0.102(0.048,0.156) p=0.002] was less than that in girls [0.394(0.352,0.437) p<0.001].

Conclusion: As expected, puberty and chronological age were strongly related to FNW (HM2), particularly in boys. In addition, puberty was associated with changes in femoral head morphology predicted to reduce subsequent OA risk (HM3), however the converse was observed with chronological age. Adolescence is an important time for hip maturation, with puberty and chronological age exerting distinct influences on those aspects potentially related to future OA risk.

P51

Investigation of relationships between hip bone mineral density and hip shape in adolescent and adult females

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Objectives: The basis for the inverse relationship between osteoporosis and osteoarthritis is unclear. In the present study, we investigated whether shared developmental influences on hip bone mineral density (BMD) and shape might contribute to this relationship, by studying cross-sectional associations between hip BMD and hip shape in adolescent and adult females.

Methods: Mothers and female offspring from the Avon Longitudinal Study of Parents and Children, a UK population based cohort, were investigated. To quantify proximal femur shape hip DXA scans were analysed in Shape software (Aberdeen, UK) using a 53-point Statistical Shape Model (SSM). Scores from an adult reference SSM (N=19379) were applied to allow comparison between mothers and children, and principal component analysis was used to generate independent modes of variation (hip shape mode (HM) scores) for each image. We examined the association of hip BMD with the top ten HMs, using multivariable linear regression adjusted for age, height, fat and lean mass. Results are expressed as unit change in outcome per unit increase in hip BMD with 95% confidence intervals.

Results: Data were available from 1036 females at mean (SD) age 13.8(0.21) years, 2438 females at mean age 17.8(0.40) years, and 4298 females at mean age 48.1 (4.2) years. Hip BMD was related to the majority of HMs, e.g. HM2 (variation in femoral neck width), HM5 (variation in femoral head size) and HM7 (variation in femoral head width) were strongly associated with hip BMD at ages 13.8, 17.8 years and in adults. HM4 (variation in femoral head width) was associated with hip BMD at ages 13.8 and 17.8 years but not in adults. Conversely, HM3 (variation in femoral head size) was related to hip BMD more strongly in adults.

Conclusion: Hip BMD is strongly related to aspects of hip shape, such as femoral head size and shape, which, in turn, may be related to risk of osteoarthritis in later life. Several of these associations are established in teenage years, suggesting that shared developmental processes may underlie both BMD and hip shape.

P52

Differential effects of aging on fracture resistance between female and male BALB/c mice

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With aging, there are deleterious changes in structural and material properties of human bone leading to an increase in fracture risk that is independent of bone mineral density (BMD). To identify targets for improving fracture resistance and not just BMD, pre-clinical models are needed in which material properties change with age. While BMD of the spine decreases with age in female and male mice, the reported age-related changes in cortical bone are primarily limited to whole-bone properties such as structural strength. We hypothesize that the primary change in mouse cortical bone with advanced aging is a loss of toughness, not strength, irrespective of sex. Therefore, we obtained 6-mo and 20-mo old BALB/c mice (n≥15/age/sex) from a colony maintained by the National Institute on Aging in the USA. The femoral mid-shaft was scanned at a 12 μm voxel size to assess cortical structure and density. Hydrated femurs were loaded to failure at 3 mm/min in three-point bending (anterior side in tension) to determine the age-related changes in mechanical properties. Age- and sex-related differences were identified using two-way ANOVA. As observed for human bone, the toughness of mouse bone decreased with aging for both sexes (interaction between sex and age: p=0.392). The estimated material strength however significantly decreased with aging for only the male mice (interaction: p=0.064). At the structural level, maximum moment was significantly higher for the 20-mo than for 6-mo old female mice with no age-related difference observed for the male mice (interaction: p<0.0001). While the moment of inertia (structural resistance to bending) was higher for the 20-mo. than for the 6-mo. mice, regardless of sex, cortical thickness increased in female and decreased in male mice with aging (interaction: p<0.0001) indicating that endosteal bone loss is more prevalent in male mice. Cortical tissue mineral density increased with aging for both sexes. The BALB/c mouse model of aging is suitable for pre-clinical assessment of manipulations that potentially affect brittleness, but sexual dimorphism should be considered when the intended target affects structural or material strength of cortical bone.

P53

Model of Support for Fracture Liaison Service Development

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Objectives: The vision of the National Osteoporosis Society (NOS) is a future without fragility fractures; to achieve this the charity's aim is to ensure that every person aged over 50 who breaks a bone is assessed for osteoporosis and managed appropriately through a Fracture Liaison Service (FLS). The NOS has developed a unique and bespoke service support model to promote adoption of FLS across the UK to prevent secondary fractures in people with osteoporosis.

Methods: In 2014 the FLS Implementation Group was convened, comprising of national and local stakeholders, clinicians and patient representatives, to develop a service support model. Online resources were developed to support FLS development and improvement, including: FLS Clinical Standards (2015); an FLS Implementation Toolkit to support providers and payers; accredited training and a new Competency Framework (2016) for fracture prevention practitioners. A specialist team of service development managers with clinical and commissioning experience offer consultation and guidance at every step of the process, from pathway development to successful funding of services. Advice is given regarding outcome measures and performance indicators, as well as effective data collection for service evaluation. These resources are all provided free of charge. Sites are offered both gap analysis to establish an objective assessment of the quality of service provision in relation to clinical standards, and in-Departmenth peer review with recommendations.

Results: The charity is currently supporting 166 sites across the UK; 83 sites are improving the quality of their services through peer support and/or commissioning assistance; and 58 sites are developing new services. 13 new services have been commissioned since commencement of the work programme, delivering new FLS provision to an additional 1.6 million people over 50. This represents £29,841,500 gross benefit for the local health economy, with 1482 hip fractures prevented over a 5-year period[1].

Conclusion: The NOS service development model of support is successful in driving forward the development and improvement of FLS across the UK. The NOS will continue in the long-term to support the development of FLS across the UK, in line with the aims of the charity.

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P54

Prostaglandin (PG) E_2 and I_2 synthases in resident bone cells

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Cyclooxygenase inhibitors block the loading-related bone formation response *in vivo*[1,2] suggesting the involvement of PGs. Further, PGE_2 release from the MLO-Y4 osteocyte-like cell line is a recognised response to mechanical perturbation, and current dogma is that osteocyte-derived PGE_2 participates in the mechanically adaptive bone modelling response *in vivo*. However, evidence to support this notion only comes from cell culture, and previous immunolocalization studies in tissue sections did not demonstrate PGE_2 in osteocytes; it was only found in osteoblasts. $cPLA_2$, involved in supplying arachidonic acid for cyclooxygenase and subsequent PGE_2 generation could similarly only be localised to osteoblasts. In contrast, $sPLA_2$ and 6-keto-PGF_{1a}, both related to PGI_2 production were localised to osteocytes and osteoblasts[3,4].

Objective: To localise PGE_2 and PGI_2 synthase in a range of skeletal tissues from the rat using anti PGE_2 and I_2 synthase antibodies.

Methods: Bones were harvested from young adult female rats, fixed and decalcified prior to chilling and storage at -20°C. 10 mm sections were cut in a cryostat prior to staining with primary antibodies. Diaminobenzidine was used to develop a coloured reaction product from horseradish peroxidase linked secondary antibodies.

Results: Consistent with previous studies: PGE_2 synthase was absent from resident osteocytes in limb bones, but present in osteoblasts. There was evidence of some positive staining in calvarial osteocytes. PGI_2 synthase was detected in osteocytes and osteoblasts.

Conclusions: We suggest that PGI_2 derived from osteocytes will participate in the osteocyte driven mechanically adaptive response. These data imply that osteocyte-derived PGE_2, however, cannot contribute to the regulation of this process. This does not preclude the involvement of osteoblast-derived PGE_2 in the mechanical response.

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P55

Characteristics of a population of postmenopausal women on a long-term bisphosphonate holiday

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Objectives: Bisphophonates (BP) are first-line treatments for postmenopausal osteoporosis but long-term treatment may cause rare but serious side-effects. Drug holidays may reduce the risks of these side-effects. We report the effect of discontinuation of BP treatment on bone turnover, bone mineral density (BMD) and fractures in postmenopausal women in an osteoporosis service setting.

Methods: We measured urinary N-telopeptide crosslink of type I collagen (uNTx) in postmenopausal women who had received alendronate for > 3 years. Second-void, morning uNTx was measured by ELISA and corrected for urine creatinine. BMD at the lumbar spine (LS) and femoral neck (FN) were measured at clinic review and follow-up using dual energy x-ray absorptiometry (Hologic QDR4500A), fracture data was also collected at these time points.

Results: 29 women (mean [SD] age 69.7[6.7] years) who received a median (range) of 8 (3-11) years of alendronate were followed-up over 4 (3-5) years. Treatment was initiated in 86.2% of women due to a diagnosis of osteoporosis, 6.9% due to a fracture, 6.9% due to steroid or aromatase inhibitor treatment. The majority of subjects stopped due to treatment length >5years (51.7%), 27.6% were no longer osteoporotic and 17.2% due to patient concerns.

56.5% of subjects were osteoporotic at the LS and 27.6% at the FN, 17.2% were osteoporotic at both sites. The LS was not significantly different from baseline at any time point measured during follow-up. In comparison FN BMD was significantly lower than baseline in Year 4 and 5 ($p>0.05$).

uNTx was significantly increased from baseline up to year 4 during the follow-up period ($p<0.05$). The largest increase in median uNTx was seen in the first year, only 1 uNTx result increased above the premenopausal reference range.

Three patients had a fragility fracture during BP treatment, all wrist fractures, while only 1 patient had a fracture during the follow-up period.

Conclusions: uNTx levels rise rapidly in the first year of a bisphosphonate holiday in response to the discontinuation of alendronate. LS BMD appears to respond at a slower rate to discontinuation in comparison to FN BMD.

P56

The roles of AKT and NHERF-1 in osteoarthritis

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NHERF-1 has been shown to be down-regulated in bone in osteoarthritis (OA) compared with osteoporosis. NHERF-1 directly interacts with PTEN, the main inhibitor of AKT, regulating cell proliferation. Down-regulation or translocation of NHERF-1 inhibits PTEN activity, increasing AKT activation and cell proliferation. The aim was to investigate the role of AKT in OA cells.

Chondrocytes and osteoblasts were isolated from tissue collected from patients undergoing elective arthroplasty for OA or hemiarthroplasty following hip fracture (control). All patients were fully consented and tissue was collected under the auspices of Grampian Biorepository. Cells were cultured under standard conditions. RNA was isolated, reverse transcribed and gene expression was analysed by qPCR. AKT1 and AKT1 pS473 protein expression was determined by ELISA. NHERF-1, PTEN and AKT1 expression and co-localisation were determined by immunofluorescence in fixed cultured cells grown on glass coverslips.

AKT1 protein expression was increased in both OA cell types compared to control cells. AKT1 pS473 phosphorylation was increased in OA chondrocytes but no difference was observed in osteoblasts. Tumour suppressor$P27^{kip}$ gene expression was decreased in both OA cell types compared to control. NHERF-1

gene expression was down-regulated in OA chondrocytes but up-regulated in OA osteoblasts compared to control. No differences were observed in AKT1 or PTEN gene expression between OA and control or between cell types. NHERF-1, AKT1 and AKT1 pS473 were partially co-localised in all cell types, with higher expression of AKT1 pS473 in OA osteoblasts. NHERF-1 and PTEN both demonstrated accumulation in the cytoplasm, with co-localisation in osteoblasts. The co-localisation was observed to be increased in both OA cell types compared to control.

AKT1 activation appears to be increased in OA tissues with increases in both native and phosphorylated forms, and decreased$P27^{kip}$ gene expression. This is consistent with the observed cytoplasmic translocation of both NHERF-1 and PTEN in OA cells. These results suggest that inappropriately increased activation of a known cell proliferation pathway may play a partial role in subchondral tissue hyperplasia seen in OA.

P57

Vertebral Fractures in children with chronic inflammatory and/or disabling conditions: the SNAP study

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Objectives: The SNAP study is a prospective fracture study of children with chronic inflammatory and/or disabling conditions. The overall study aim is to assess causal links between body-size adjusted bone density and low trauma fracture.

Methods: 330 children aged 5-18 years were recruited from 7 disease groups namely; acute lymphoblastic leukaemia (ALL), rheumatological disease, inflammatory bowel disease, cystic fibrosis, coeliac disease, Duchenne muscular dystrophy (DMD) and cerebral palsy. At baseline, bone density by DXA (lumbar spine [LS BMAD] and total body less head [TBLH BMD]), forearm pQCT (trabecular density at the 4% site [Trab vBMD], and ratio of bone/muscle area at the 66% site [Radius BA/MA], hand radiographs (Bone health index [BHI], BoneXpert), lateral spinal radiographs and medical history were assessed. A threshold of Z-score<-2.0 was set to dichotomise the bone density Z-scores and used in conjunction with a binary prediction model to assess diagnostic accuracy.

Results: Spinal radiographs identified 71 children (21.5%) with vertebral fractures, with highest incidence for children with ALL (26/51) and DMD (14/42) ($p<0.001$). Steroid exposure, back pain and immobility were reported in 50%, 37% and 14% of patients, respectively. Bone density Z-scores were significantly lower in the fracture group for LS BMAD, Trab vBMD, Radius BA/MA, and BHI. The variables most predictive of vertebral fracture were Trab vBMD (OR 3.4 (1.6-7.0), BHI (OR 2.8 (1.6-4.9) and BA:MA (OR 2.3 (1.0-5.3) ($p<0.05$), with corticosteroid exposure (OR 1.7 (1.0-2.9) and back pain (OR 1.7 (1.0-2.8) also significant.

Conclusion: Disease itself, back pain and corticosteroid exposure are significantly associated with risk of vertebral fracture. However, the variables most predictive of vertebral fracture were low trabecular density measured by pQCT and BHI by BoneXpert. Evidence of the predictive power of these measurements will only be confirmed with future follow-up of this group.

P58

Body mass index across adulthood and spine shape in early old age in a UK birth cohort

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To investigate associations between BMI across adulthood and spine shape at age 60-64 years in participants from the MRC National Survey of Health and Development (NSHD).

1601 individuals from the MRC NSHD, born in the same week in 1946, had a DXA scan of the lumbar spine between age 60 and 64 years. 72 images were excluded due to poor quality, leaving 1529 images for analysis. Spine shape was described using an 89-point template. Points were entered into a statistical shape model using Shape software (University of Aberdeen), and mean spine shape and independent modes of variation in shape were identified using principal components analysis. Shape Modes (SM) 1-4 describing 78.7% of total variation in spine shape were analysed. BMI was measured at ages 15, 36, 43, 53 and 60-64 years and self-reported at ages 20 and 26 years. Sex-stratified linear regression models were used to investigate associations between BMI at each age and each SM.

In women, higher BMI at ages 20 and 43 were associated with lower SM2 scores (with negative scores describing snaking and uneven spine curvatures) [for example, the mean difference in SM2 scores per 1 kg/m^2 increase in BMI at age 20 =-0.04(95%CI -0.07, -0.01)]. In men, similar associations were found between higher BMI at most ages and SM2 scores. Similarly, higher BMI at all ages were associated with lower SM3 in both sexes (with negative scores describing large anterior-posterior vertebral diameter) [for example, the mean difference in SM3 scores per 1 kg/m^2 increase in BMI at age 20=-0.10(95%CI -0.13, -0.06) among men]. Conversely, higher BMI from ages 15 to 43 were associated with higher SM4 scores in women (with positive scores describing a more uniform curvature with larger lumbar disc spaces). No associations were observed between BMI and SM1, which described the overall spinal curvature.

BMI across adulthood was associated with specific features of spinal shape. These results will enable future studies exploring spine shape and low back pain to include the association with life-course BMI as a factor.

P59

Cytokine and Hormonal Regulation of Bone Marrow Immune Cell Wnt1Ob Expression

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Wnt1Ob is a crucial regulator of bone density via promoting osteoblastogenesis. Though parathyroid hormone has been shown to regulate Wnt1Ob expression in $CD8^+$ T cells, the relative expression and other source(s) of Wnt1Ob in bone marrow immune

cells (BMICs) is not known. Sex hormones and cytokines including, estrogen and TNFα are critical regulators of bone physiology. Whether estrogen and TNFα regulate BMIC Wnt1Ob expression is unclear. To determine the potential regulation of Wnt1Ob by estrogen and TNFα, we used flow cytometry to assess Wnt1Ob expression and Wnt1Ob+ numbers in BMICs under estrogen- and TNFα-deficient conditions.

Effect of TNFα on Wnt1Ob expression was determined in male and female C57BL/6 wildtype and TNFα knockout mice. Effect of estrogen on Wnt1Ob expression was investigated 4, 6 and 8 weeks post-surgery in ovariectomized Balb/c mice. Intracellular Wnt1Ob was detected using goat anti-mouse Wnt1Ob and donkey anti-goat IgG-CFL 488 secondary antibody and analyzed by flow cytometry. Results were expressed as Wnt1Ob signal/cell and the number of Wnt1Ob$^+$ cells. BMICs were differentiated using specific markers: CD3, CD4, CD8, F4/80 and CD11c.

Wnt1Ob expression was sex-specific with 1.8-fold higher Wnt1Ob signal in females. Analysis of cell types revealed lineage differences; females exhibited an elevated percentage of Wnt1Ob$^+$ myeloid cells (8.9% Vs 5.4%) and male's having higher Wnt1Ob$^+$ lymphoid cells (6.3% Vs 2.5%). Regulation of Wnt1Ob by TNFα further revealed sex-specific differences. Ablation of TNFα increased male total BM Wnt1Ob expression 1.5-fold, no effect was observed in females. Conversely, in males, TNFα ablation significantly reduced numbers of BM Wnt1Ob$^+$ CD4$^+$ T cells (65%), CD8$^+$ T cells (59%), dendritic cells (59%), macrophages (56%) and granulocytes (52%) but only had modest effects in females. In contrast to TNFα, estrogen-deficiency had indirect effects on BMIC Wnt1Ob levels; reducing the average percentage of BM Wnt1Ob$^+$ CD8$^+$ T cells by 25% and granulocytes by 26% across an 8-week time course.

Our results demonstrate unique cell type- and sex-dependent effects on BMIC Wnt1Ob expression. Together, our results reveal previously unidentified BMIC sources of Wnt1Ob under complex hormonal and cytokine regulation.

P60

Drinking green tea alleviates alveolar bone resorption in ligature-induced periodontitis in mice

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Background and Objective: Green tea is widely accepted for its beneficial properties on human health, including anti-bacterial, anti-inflammatory and anti-oxidative effects. The present study was investigated whether drinking green tea has an inhibitory effect on bone resorption in ligature-induced periodontitis in mice models.

Material and Methods: Sixty-six C57BL/6 eight-week-old male mice were fed by normal food, administered with Japanese green tea and sterile distilled water. Periodontitis was induced by ligature for 7 days in upper left maxillary molar and left un-ligated on contralateral side for control. After removing ligation, green tea in different concentration (1.5 g/60 ml, 3 g/60 ml and 6 g/60 ml) were treated orally. Up to next 1 and 2 weeks of administration, all mice were killed. Alveolar bone loss was evaluated by Micro-CT detection in buccal and palatal sides both area and distance methods. The number of inflammatory cells and osteoclasts were carried out histopathologically.

Results: In ligated side, alveolar bone resorption was decreased significantly in buccal side but not significantly in palatal side dose-dependently both area and distance methods. Moreover, in un-ligated side, alveolar bone loss in buccal side of area method was significantly decreased in all concentration of drinking green tea. Meanwhile, in distance method, only 3 g/60 ml and 6 g/60 ml groups were presented alleviation of alveolar bone resorption significantly. At the first week of green tea administration, histological analysis showed a lower number of inflammatory cells considerably in all concentration. Furthermore, merely the 6 g/60 ml groups had no difference of inflammatory cells compared to the un-ligated side. In addition, a significantly decreased number of osteoclast were observed in 6 g/60ml groups both 1 and 2 weeks.

Conclusion: These findings demonstrate that systemic administration of drinking green tea could have a therapeutic effect on alveolar bone resorption in term of concentration & time-dependence in experimental periodontitis in mice models.

P61

Longitudinal differences in bone development in adolescent male athletes: The PRO-BONE study

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Objectives: Exercise can optimise bone development during growth however, weight bearing and non-weight bearing sports may different effects on bone development. This study aimed to investigate the longitudinal differences in bone acquisition and bone metabolism between adolescent males participating in osteogenic (football) and non-osteogenic (swimming, cycling) sports compared to a control group over 1 year.

Methods: In the present study 116 adolescent males (13.1±0.1 years: 37 footballers, 37 swimmers, 28 cyclists engaged in these sports more than 3 hours per week in the last three or more years and 14 controls not engaged in these sports more than 3 hours per week in the last or more three years) were measured at baseline and after 1 year of sports specific training. Dual-energy x-ray absorptiometry (DXA) assessed bone mineral content (BMC) at whole body, lumbar spine and dual femurs. Quantitative ultrasound (QUS) assessed bone stiffness. Hip structural analysis (HSA) and trabecular bone score (TBS) assessed bone geometry estimates and trabecular structure respectively. Blood markers analysed for procollagen type 1 aminoterminal propeptide (P1NP), Carboxi-terminal telopeptide of type 1 collagen (CTX-I) and 25 hydroxyvitamin D [25(OH)D]. Moderate to vigorous physical activity (MVPA) was measured for 7 days using accelerometers. Results were adjusted for age, height, lean mass, MVPA and baseline bone outcomes.

Results: Longitudinal participation in football was associated with significantly higher adjusted BMC acquisition at the total body, total hip, shaft, Ward's, legs, lumbar spine and femoral neck compared to cyclists. Also, footballers had significantly higher adjusted BMC acquisition at total body, shaft, legs and lumbar spine compared to

swimmers. Footballers had significantly higher acquisition in all HSA outcomes and bone stiffness compared to cyclists, and significantly higher acquisition in TBS score at the lumbar spine compared to cyclists and swimmers. After 1 year footballers had significantly higherP1NP compared to swimmers and cyclists, and 25(OH)D was significantly higher in footballers and cyclists compared to controls and swimmers.

Conclusion: This novel longitudinal study shows that one year of football participation was associated with significantly greater bone development compared to cycling and swimming, suggesting that programmes to improve bone development in adolescent males engaged in non-osteogenic sports.

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P62 - Abstract withdrawn

P63

A case of osteonecrosis of the jaw in paget's disease of bone

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Text In 2015 an 84 year old lady with Paget's disease of bone was referred to the the bone clinic at James Cook Univeristy Hospital from another hopsital for advice on further treatment of what was said to be Pagetic bone pain in the right hip. She had polyostotic disease affecting pelvis and left femur. Twenty years previously in 1997 she had been diagnosed with breast cancer for which she had a mastecomy, radiotherapy and tamoxifen. She was complaining of right hip pain and imaging at that time revealed sclerotic bone in the pelvis and isotope bone scan revealed uptake in the pelvis and left femur. This was labelled as being due to metastatic breast cancer. She was given pamidronate monthly and the pain improved. Over time it became clear that this was in fact Paget's disease of bone. As the pain kept relapsing they repeated the pamidronate. A regime of monthly pamidornate was the entered into for the next 17 years. In the last couple of years at that was changed to monthly 3 mg of zoledronate. In 2014 she noticed bits of bone in her mouth coming from her left maxilla. This was found to be due to onsteonecrosis of the jaw confirmed om imaging and biospy. She has been left with a permanent whole in her left maxilla. As bone persisted in the right hip and bisphosphonates could no-longer be given she was referred to the author. Her blood tests showed the alkaline phosphatase to be 85. Examination found that the the rigth hip had extremely limited range of movement, which caused a lot of pain. X-rays confirmed teh rpesence of advanced secondary osteoarthritis. Subsequently, a right total hip replacement was performed with no significant blood loss. The hip pain was cured and 2 years later the lady remains well with an alkaline phosphatase of around 85.

P64

Satoyoshi syndrome: an unusual cause of osteomalacia

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A 24 year old South Asain married lady was referred to the bone clinic beacuse of estremely low bone density with a T-score of -2.7. A DXA scan had been performed because of hypogonadism secondary to autoimunne oopheritis. She was also known to have beta-thalasaemia trait and alopecia totalis. She had been palced on HRT. A full osteopororitc screen turned up no other secondary causes for low BMD other than the fact that she had osteomalcia. This was corrected with calcium and vitamin D supplemnetation giving 8OOIu of choleclaciferol. Soem 6 months later she remained insufficient and even with large doses of supplemnatio the vitamin D could not be brought above 34nmol/L. Over the next 2 yeras she devloped raipdly worsening arm and leg cramps and was eventually diagnosed as stiff person syndrome by the neurologists. This improved with phenytoin. She also devloped probelms with weight loss and subsequently diarrohea. This was extebsively investigated by the gastroenterologists, but no cause could be found. She was rapidly waisting away, nutrition became impossible and she was evetually admitted to hospital in extremis. NG feeding was unsuccesul and she was being fed parenterally. Endoscopy and colonoscopy were undertaken. the biopsy findings were of mild , patchy inflammatory changes. The patient was reviewed by the authors and close liison between rheumatology and pathologists came to the diagnosis of satoyoshi syndrome. The is an extremley rare condition with only 40 or so cases reported since 1967. It is an automimmune condition characterised by: stiff person syndrome, alopecia totalis, autoimmune oopheritis and malabsorption secondary to the inability to digest carbohydrate. The lady was treate with intravenous methylprednisolone and then oral prednisolone. This brought about resolution of her symptoms. She was able to eat normally and gained weigtht. The prednsiolone was gradually weaned down over montsh and maintenance therapy with azathioprine was begun. Over time her menstruation retarted, viatmin D levels normalised and hair began to regrow. Her BMD returned to nromal and she made a full revovery. In 2015 she became pregnant and delivered a healthy boy.

P65

Gut microbiota and bone metabolism

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The gut microbiota (GM), the commensal bacteria living in our intestine, performs numerous useful functions, including modulating host metabolism and immune status. Our recent studies demonstrate that the GM is also a regulator of bone mass and we propose that the effect of the GM on bone mass is mediated via effects on the immune system, which in turn regulates osteoclastogenesis. A role of the GM in bone metabolism is further supported by studies demonstrating that antibiotic, probiotic, and prebiotic treatments that impact GM composition regulate bone metabolism. Collectively, these studies suggest that the GM may be a novel therapeutic target for osteoporosis. Treatment with probiotics has already been shown to improve bone mass in rodent models of bone loss, but future randomized clinical trials are required to determine the possible effect of probiotics and other novel therapies modulating the GM composition on bone mass and fracture risk in patients with osteoporosis.

Access to cheaper sequencing and improved bioinformatics tools will allow metagenomic sequencing for the analysis of the GM composition in large prospective clinical cohort studies. This can be used to evaluate the predictive value of the GM composition

as a biomarker for low bone mass and fracture risk. In addition, metatranscriptomics and metaproteomics will most likely be used to identify the microbial genes and proteins that have an impact on bone mass and fracture risk.

We propose a new cross-disciplinary GM-bone research field called 'osteo-microbiology', bridging the gaps between bone physiology, gastroenterology, immunology, and microbiology. Future studies are clearly warranted in this new research field to determine if the GM composition might be used as a biomarker for fracture risk prediction and to validate the GM as a possible novel therapeutic target for osteoporosis.

P66

Running on time: the role of the circadian clocks in the musculoskeletal system

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The night and day cycle governs the circadian (24 hourly) rhythm of rest/activity, physiology and metabolism in animals and humans. A central clock in the brain coordinates the rhythmic locomotion behaviour and synchronizes various local oscillators, such as those found in the musculoskeletal system. Disruptions to circadian rhythms (e.g. during ageing) have been linked to various diseases. Osteoarthritis and low back pain are among the most prevalent skeletal conditions associated with old age. However, the reasons why susceptibility to these diseases increases with age are not well understood. Work from our group has revealed a functional link between circadian clocks and the homeostasis of the articular cartilage and intervertebral disc tissue. We show that the daily rhythms in these tissues become dampened and out-of-phase during ageing. Moreover, mice with targeted knockout of an essential clock gene (Bmal1) in chondrocytes and disc cells have profound, yet tissue-specific degeneration. This new avenue of research holds potential to better understand the pathogenesis of these skeletal disorders.

P67

Fracture toughness of cancellous bone as a function of BV/TV and its microarchitecture

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We have recently introduced fracture toughness tests to assess the structural integrity of cancellous bone specimens from OP and OA patients. FT refers to stress field conditions to start growing a crack and thus relates to the failure resistant behaviour. Recently we have shown that FT depends primarily on BV/TV (Cook & Zioupos 2009; Cook et al. 2010), but it may also depend on microarchitecture (mA) (Greenwood et al. 2015). The quantitative interplay between these two (BV/TV, mA) has never been explored before and it may reveal valuable insights for the fracture behaviour of cancellous bone for prediction and diagnosis.

Sixty-one samples were taken from 37 OP and 13 OA patients ranging from ages 59-96 years. The specimens were disk-shaped (ASTM plane-strain fracture toughness standard E399-9). The samples were µCT scanned and various morphological parameters were determined. Morphological parameters along with BV/TV were used in stepwise multiple regression analysis to produce models for FT. In this study we have two primary objectives to investigate the use of predictive models for FT, and to look more particularly, if there are any orientation effects for cracks growing across (A_C) and along (A_L) the trabeculae.

Multiple regression models showed that if microarchitectural parameters are added to BV/TV the predictive power increases significantly. Kc on BV/TV alone had maximum R^2 0.66; and after adding mA parameters in stepwise multiple regression regardless of direction and then along/across trabeculae it was raised to R^2 0.79. This is not unexpected and has been hypothesised for years especially as cancellous bone shows certain microarchitectural alterations with age and disease. However, what is more important in our findings is the fact that predictive analysis depended strongly on direction. In the A_L loading configuration, no inclusion of any microarchitectural parameter could statistically improve the predictive power of the models suggesting that trabecular architecture is optimised and fashioned in such a way as to resisting fracture in a specific direction.

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P68 - Abstract withdrawn

P69

The relationship between circulating adiponectin and leptin with bone mineral density (BMD), arterial calcification and stiffness: a cross-sectional study in post-menopausal women

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Background: Adiponectin and Leptin may be involved in the underlying mechanisms linking osteoporosis and cardiovascular disease (CVD).

Objective: To explore the relationship between circulating adiponectin and leptin with bone mineral density (BMD), arterial stiffness and vascular calcification (VC) in post-menopausal women.

Design: We studied 386 ambulant community-dwelling postmenopausal women aged (mean [SD] 61 [6.4] years. BMD at the lumbar spine (LS), femoral neck (FN), and total hip (TH), body composition; fat mass (FM) and lean mass (LM) as well as abdominal aortic calcification (AAC) were determined by dual energy X-ray absorptiometry (DXA). Pulse wave velocity (PWV) and augmentation index (AI), markers of arterial stiffness were measured. Fasting adiponectin and leptin were quantified in serum.

Results: A negative association was seen between adiponectin and BMD at the FN in women with osteoporosis adjusted for age, FM, LM and lifestyle variables ($p=0.037$). Serum adiponectin was significantly higher in women with fractures (20.8 [9.3] µg/ml compared to those without (18.5 [8.6] µg/ml, $p<0.018$) and associated with a significant increased risk of fracture (HR 1.033, 95% CI 1.004-1.064, $p=0.027$).Adiponectin was independently associated with AAC ($p=0.014$) and significantly higher in women with AAC scores >1; (19.2[9.2]) compared to those with no or low

AAC scores (<1); 16.8 [8.0], *p=0.018*). Leptin was not associated with BMD or fracture risk after adjustment. In adjusted analyses, AI was negatively associated with serum leptin (*p=0.005*).

Conclusion: Adiponectin and leptin influence bone and vascular health and may explain, partly, the observed association between osteoporosis and CVD.

P70

Missed opportunities: What the FRAX?

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Introduction: FRAX is a fracture risk assessment tool to evaluate the ten year probability of bone fracture risk.

Objective: To correlate pre-morbid FRAX score with pre admission treatment for osteoporosis in elderly patients admitted with a fragility fracture of the hip.

Methods: All patients who were admitted to University Hospital Limerick (UHL) with a fragility fracture of the hip from July to October 2016 were assessed for correlation between pre morbid hip fracture risks using FRAX and prior diagnosis or treatment for osteoporosis by primary care in the community. Electronic patient records were used to record pre-admission demographics, medications and prior diagnosis of osteoporosis.

Results: 63 patients were admitted to UHL with a fragility fracture of the hip during the study period. Of these, 40 patients were included in the study. 28 patients were female, median age was 81 years old. 13 patients (32.5%) had a FRAX score in high risk category for developing hip fracture and 17 patients (42.5%) were in the intermediate risk category. Of these 30 patients in high and intermediate risk category, only 9 (30%) were on treatment for osteoporosis prior to admission, either with calcium/vitamin D supplement, or bone protection medication, or both. 10 patients were in the low risk category as per FRAX score.

Conclusion: 75% of hip fracture patients in this study were considered intermediate or high risk of sustaining a bone fracture based on pre-morbid FRAX assessment. Only 30% of these patients were treated for osteoporosis prior to admission. There is a role for the use of FRAX in the primary care setting to identify risk and guide commencement of bone protection medication in frail elderly patients.

P71

Principal component-derived bone density phenotypes and genetic regulation of the pediatric skeleton

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Objectives: Determine if genetic variants associated with principal component-derived areal bone mineral density (aBMD) loading scores.

Methods: Our sample comprised 1,293 children of European ancestry enrolled in the longitudinal Bone Mineral Density in Childhood Study (52% female). The participants completed up to 7 annual visits. Sex and age-specific aBMD Z-scores were calculated for total hip, femoral neck, spine and distal radius. Principal components analysis, applied to the four Z-scores, generated new integrated aBMD phenotypes. Linear mixed effects models, adjusted for age, Tanner, BMI-Z, dietary calcium and physical activity, were used to test associations between a genetic score (percentage aBMD-lowering alleles carried at 63 GWAS-implicated loci) and the loading scores. We also performed a GWAS, using the baseline data, to identify loci associated with the loading scores.

Results: Four principal components (PC1-PC4) were identified that explained 68.1%, 18.6%, 10.5%, and 2.8% of the variance, respectively. A higher PC1 loading score indicated higher bone Z-scores across all four sites. The genetic score was associated with lower PC1 loading score (beta=-0.05, P=3.9x10-10); from the GWAS, rs114260199 (LMO2/CAPRIN1, P=3.9x10-8) and rs75321045 (ZMAT4, P=2.5x10-8, females) were associated with PC1 loading score. A higher PC2 loading score indicated higher distal radius Z-score only. The genetic score was not associated with PC2; from the GWAS rs67991850 (CPED1, P=2.5x10-11) was associated with PC2 loading score. A higher PC3 loading score indicated higher spine Z-score only. The genetic score was not associated with PC3; from the GWAS rs58649746 (RAB11FIP5, P=4.8x10-9, females) was associated with PC3 loading score. A higher PC4 loading score indicated lower total hip Z-score, but higher femoral neck Z-score. No genetic associations were observed for PC4.

Conclusion: We identified non-site-specific (PC1), distal radius-specific (PC2) and spine-specific phenotypes (PC3). An established genetic bone fragility score associated with the non-site-specific phenotype, but not the site-specific phenotypes. Novel variants near LMO2/CAPRIN1, ZMAT4, and RAB11FIP5 associated with non-site specific or spine specific phenotypes. These results highlight the utility of an integrated skeletal site phenotyping approach, which may help identify additional genetic loci associated with skeletal development.

P72

Modelling Stickler syndrome in zebrafish

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Objective: Stickler syndrome is a hereditary connective tissue disorder caused by defects in collagen. It is characterised by skeletal, orofacial, visual and auditory abnormalities such as scoliosis, hearing loss, retinal detachment and premature osteoarthritis. Defects in ColXI have been associated with autosomal dominant Stickler syndrome, whereas defective ColIX has been linked to autosomal recessive Stickler syndrome. Both collagen XI and IX are expressed in the notochord and otic vesicle, but only ColXI is found in the jaw region of larval zebrafish, with ColIX found in the eye. We have zebrafish lines with mutations in these genes which will

allow us to assess the suitability of zebrafish as a Stickler syndrome model, thus enabling us to study the impact of these mutations on skeletal, auditory and ocular morphogenesis.

Methods: Larval fish from Col9a1b and Col11a2 mutant lines were fixed at 3, 5 and 7 days post fertilisation and at juvenile stages, with immunohistochemistry was used to visualise the skeleton and otic vesicle. The jaw region of live transgenic larvae was imaged by confocal microscopy, and photoconversion of chondrocytes allowed tracking of structural development in jaw cartilages and joints.

Results: Preliminary results suggest that mutations in Type IX Collagen cause an eye phenotype and that Type XI Collagen mutations result in morphological abnormalities to the jaw, spine and ribs of developing zebrafish. These changes are accompanied by alterations to collagen organisation and changes to skeletal cell behaviour.

Summary and Conclusion: Our preliminary results indicate that mutations in genes associated with Stickler syndrome alter spine, rib and craniofacial morphology of zebrafish, potentially through disruption of collagen deposition. Further understanding how these mutations affect morphogenesis by altering cell behaviour is crucial for modelling Stickler syndrome in zebrafish. We plan to use this model to further explore how mutations in collagen affect its secretion and assembly, and how the resulting skeletal morphology affects susceptibility to, and age of onset of, osteoarthritis.

P73

Marine organism-derived extracts for bone growth: the discovery of novel osteogenic compounds

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Objectives: Marine extracts have previously shown osteogenic potential, and may therefore be a source of novel medical treatments for musculoskeletal conditions. Specifically, extracts containing factors capable of stimulating osteoblast activity are sought, to aid in addressing the large health care burden caused by osteoporosis, complex fracture and other bone disorders[1]. This study aimed to determine (i) the toxicity, proliferation and differentiation effects of marine extracts on primary bone marrow derived mesenchymal stem cells (hBMSCs) and (ii) extract effects *in vivo*, using a zebrafish bone formation model.

Methods: Extracts were produced from a variety of Irish marine invertebrates. Briefly, raw material was treated with dichloromethane and methanol to produce both dissolved and undissolved residue fractions. The osteogenic potential of these fractions were tested *in vitro* with primary bone marrow derived mesenchymal stem cells (hBMSCs). Toxicity (LDH assay), cell viability (XTT), proliferation (crystal violet) and differentiation (alkaline phosphatase) were all determined. Promising extracts were then tested *in vivo*, using a larval (operculum based) and adult (caudal fin) zebrafish bone formation model[2].

Results: Of the approximate 120 extracts screened to date, most were not cytotoxic - though few stimulated increases in activity either. The most promising exception to this were algae powder residues, the material retained after DMC/methanol extraction, which were non-toxic and active both *in vitro* and *in vivo*. Taking the red algae *Plocamium cartilagenum* as an example, concentrations less than 30 µg/ml elicited a two-fold increase in hBMSC proliferation and differentiation. *In vivo, P. cartilagenum* was also shown to significantly and repeatedly increase operculum (gill covering) size. Whereby, growth was consistently 50% greater than that of the vehicle control and also exceeded bone formation with vitamin D positive controls.

Conclusion: This work demonstrates the osteogenic potential of Irish marine invertebrate extracts. Chemical analysis of the fractions is now required to identify active compounds.

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P74

Ionic mechanisms in adipogenic differentiation of osteoblastic cells treated with ghrelin

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Ghrelin is a gut-derived peptide that has been shown to promote osteoblast proliferation and differentiation[1]. Our preliminary data indicate that ghrelin is able to at least partially inhibit the adipogenic differentiation of 7F2 cells, an osteoblastic cell line derived from$P53^{-/-}$ mice. Furthermore, cell membrane potential controls differentiation of osteoblasts and adipocytes[2], and a potassium channel, the BK channel, has been shown to affect mineralisation in human osteoblasts[3]. In this study, we aimed to identify ion channels in 7F2 cells treated with basal or adipogenic medium, in the presence or absence of ghrelin.

7F2 cells were induced to undergo adipogenic differentiation by adding dexamethasone, indomethacin and ascorbic acid to the basal medium, supplemented with ghrelin or not. We used RT-PCR, qPCR and single-channel patch-clamping to detect the presence of several ion channels, with a particular focus on potassium channels.

RT-PCR showed the presence of a subunit of the BK channel KCa1.1, and the presence of inwardly-rectifying subunit Kir6.1 and its associated subunit SUR2B. Interestingly, KCa1.1 mRNA expression seemed to be down-regulated by adipogenic medium. Preliminary patch-clamp results indicate the presence of various types of currents, including inwardly-rectifying currents. BK channel activity was also detected, particularly in 7F2 cells cultured with basal medium.

Our next experiments will consist of further characterising ion channels in 7F2 cells using whole-cell patch clamping. We will also investigate the role of ionic mechanisms in the adipogenic differentiation of 7F2 cells, using pharmalogical modulation of cell membrane potential, and testing whether ghrelin affects ion channels when added to the medium. These results will provide a better understanding of the mechanisms underlying pathological adipogenesis in bone.

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P75

Modelling osteoarthritis and other adult skeletal conditions in zebrafish

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Objectives: A majority of people will suffer from either osteoarthritis (OA) or from back pain at some point in their lives and radiography often reveals changes to limb joints and the vertebral column including reduced joint spacing, intervertebral disc collapse and osteophyte formation. Despite the frequency of these degenerative conditions, many of the molecular and genetic changes that underpin such changes to the skeleton remain unclear as does the role played by the OA susceptibility genes identified through Human Genome Wide Association studies (GWAs) on skeletal cell behaviour.

Methods: High resolution Micro Computerised Tomography (microCT) scanning, and contrast enhanced microCT scanning (to visualise soft tissues) were used to study the skeleton of ageing wild type zebrafish. Picrosirius red staining visualised with polarised light microscopy and Second Harmonic Generation with a multiphoton microscope was used to reveal collagen alignment. Immunohistochemistry for a number of skeletal proteins and proteoglycans as well as various histological stains was used on sections from ageing fish. We examined genetically wild type fish and fish carrying mutations in OA susceptibility genes Chst11, Col9a1, Col11a2 and Gdf5.

Results: We show that deformities that are externally visible are accompanied by radiographic changes to the skeleton. These include misalignment of joints, formation of osteophytes and changes to local bone density. We also show that ageing fish show changes to cartilage and bone ultrastructure and express various proteoglycan epitopes that correlate with the severity of the phenotype. We show that carriers of mutations for OA susceptibility genes show increased frequency and severity of skeletal changes, which are underpinned by earlier changes to cell specification.

Discussion: We demonstrate that skeletal deformities, such as joint misalignment, intervertebral disc collapse and osteophyte formation strongly resembling those seen in other fish species and humans, occur in ageing zebrafish. Zebrafish with their genetic amenability and the increasing availability of imaging tools for adult fish, therefore, provide a good model to probe the genetic and molecular changes that underpin changes to the ageing skeleton.

P76

Incubation at physiological temperature promotes ovine osteoblast proliferation and activity

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Sheep are a model organism used in orthopaedic research to understand osteoporosis, fracture healing and biomaterials for bone implants. Temperature is an important factor influencing cell growth and activity. Sheep have a core temperature between 38.3-39.9ºC so our main goal was to determine optimal conditions for sheep osteoblast functional studies. We hypothesised that ovine osteoblasts have optimal growth and activity when cultured at 38.5°C.

Bone fragments were isolated from the trabecular region of the femoral head from a 3 year old sheep. After washing and trypsin/collagenase digestion the fragments were seeded in DMEM at 37°C to allow outgrowth of cells. Once confluent $1.3x10^4$ cells/cm^2 cells were seeded (n=6) in 24 and 6 well trays. Cells were incubated at 37°C or 38.5ºC with or without 50 µg/ml of ascorbate. After 4 days we determined cell proliferation with crystal violet staining. After confluence, cells at both temperatures were stimulated with 100µg/ml ascorbate+2mM β-glycerophosphate and with or without 10nM of dexamethasone. TNAP activity was determined after 7 days in mineralization media.

Cultures with ascorbate had an 18% increase in proliferation at 37ºC (p=0.0003), but addition of ascorbate had no effect at 38.5ºC. Osteoblasts cultured at 38.5ºC had increased proliferation both with (12%, p=0.0119) and without ascorbate (4%, p=0.0385) when compared to 37°C.

After 7 days in mineralization media, TNAP activity was increased in all culture conditions at 38.5ºC, when compared to 37°C. Cells that were cultured with ascorbate in the first days at 38.5ºC and mineralizing with the addition of dexamethasone had the highest TNAP activity when compared to all remaining conditions (27 to 64% increase, p<0.0001).

Our data indicates that ovine osteoblasts cultured at 38.5ºC with ascorbate in the initial phase show the highest proliferation and that addition of dexamethasone is important for TNAP activity and consequent mineralization at both 37 and 38.5°C. This work shows the importance of adjusting incubator temperature to animal core temperature when working with primary cells *in vitro*.

P77

Brittleness phenotype of TallyHO mice does not worsen with duration of type 2 diabetes

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Type 2 diabetes (T2D) is a risk factor of fracture susceptibility that occurs without a reduction in areal bone mineral density. Fracture risk increases as diabetes duration increases in adults potentially because of deterioration in material properties of bone. With rising rates of juvenile T2D, a pre-clinical model to study long-term effects of diabetes on bone is needed. We hypothesized that an increase in duration of T2D facilitates a progressive loss in fracture resistance in a mouse model of juvenile T2D. TallyHO mice and SWR/J (non-diabetic) controls (n≥12/strain/age) were purchased from Jackson's Laboratory in the USA. Upon sacrifice at 16-wks and 34-wks of age, left and right femurs were collected, evaluated using microcomputed tomography (µCT), and then loaded at 3 mm/min (intact) or at 0.6 mm/min (notched), respectively, in 3-point bending. ANOVA/ANCOVA was used to determine whether material and structural properties were significantly different between strains (effect of diabetes) and age without/with body weight (BW) included as a covariate. BW was higher in TallyHO than in SWR/J mice as early as 5-wks. TallyHO mice were hyperglycemic, on average, starting at 8-wks. Bending material strength (independent of structure) was higher for TallyHO than for SWR/J bones at 16-wks (p<0.0001), but there was no diabetes-related difference at 34-wks (p=0.462). Toughness (intact) was lower for TallyHO than for SWR/J mice at both 16-wks (p=0.014) and 34-wks (p<0.0001), though this brittleness did not progressively worsen with age or duration of

T2D. Fracture toughness (notched) did not differ between strains but decreased with age in both strains (p_{age}=0.023). At the whole-bone level (dependent on structure), maximum moment was higher for the TallyHO than for SWR/J bones and increased with age in both strains (p_{strain}<0.0001, p_{age}<0.0001). Diabetes-related differences in structural strength, material strength, and toughness were not independent of BW. For whole-bone structure, porosity was lower (p_{strain}<0.0001) while cortical area and tissue mineral density were higher for TallyHO mice than for non-diabetic controls, regardless of age (p_{strain}<0.0001 and p_{strain}<0.0001). Overall, loss in fracture resistance did not worsen with duration of diabetes.

P78

Systemic administration of rosiglitazone stimulates apoptosis of osteocytes, interfering in the development of induced periapical lesions in mice

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Objective: To evaluate a protocol for systemic administration of Rosiglitazone in mice in order to stimulate the apoptosis of osteocytes in the jaws and to evaluate the effect of osteocytes apoptosis induced by Rosiglitazone in the progression of periapical lesions in mice at 7, 21 and 42 days.

Methods: C57BL/6 mice at 4 to 5 weeks of age were used. In phase 1, mice (n=24) were treated with Rosiglitazone (gavage, 10 mg/kg dose) or without (PBS+10%DMSO) for 1, 2 or 3 weeks. We used TUNEL and DAPI methods for quantification of apoptotic cells. In phase 2, mice (n=30) received Rosigitazone for 2 weeks or just vehicle for 1 week (n=30) and periapical lesions were induced for 7, 21 or 42 days. We performed the measurement of periapical lesions; tartrate-resistant acid phosphatase staining (TRAP), dual-energy x-ray absorptiometry (DXA) for evaluation of bone mineral density (BMD) in long bone and gene evaluation by qRT-PCR of osteocytes markers (*Sost, Hyou1* and *Dmp1*).

Results: It was observed that the systemic administration of Rosiglitazone for 2 weeks showed the apoptosis of osteocytes in a more expressive manner. In phase 2, in the groups which received Rosiglitazone, a trend toward larger periapical lesions was observed (p>0,05). Rosiglitazone also induced a greater number of osteoclasts and greater expression of *Sost* and *Hyou1* at 21 days of lesion. Moreover, there was not statistically significant differences in *Dmp1* expression nor in the femurs BMD.

Conclusions: Rosiglitazone stimulated the apoptosis of osteocytes interfering in the progression of periapical lesions in mice.

P79

The entheses of the rotator cuff, bone mineral density (osteoporosis) and occupations

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Objectives: To test the impact of bone mineral density and metacarpal cortical index on entheseal changes in the rotator cuff. These changes, visible on dry bone, are known increase in frequency with age: this effect may be linked to lower bone mineral density.

To determine whether manual labour reduces risk of osteoporosis, by increasing maximal bone mineral density.

Methods: Male skeletons from the Coimbra identified skeletal aged 20+ were recorded for entheseal change presence using the new Coimbra method for recording (n=249). These skeletons have known sex, age-at-death, and occupation and lived between 1826-1938. Occupations were categorised following standard methods. Radiogrammetry in a subset of these individuals (n=147) was used to calculate the second metacarpal cortical index. In a smaller subset (n=126) bone mineral density was measured in the left proximal femur using dual x-ray absorptiometry at several sites (dry rice acted as a soft tissue substitute).

Results: Logistic regression showed no effect (p<0.05) of occupation on total bone mineral density, bone mineral density at the femoral neck or metacarpal cortical index. Total bone mineral density was found to have an effect on some types of entheseal change presence, but this was not statistically significant when age was taken into account. Metacarpal cortical index was found to have a minimal impact on some entheseal changes: mineralisation (Chi^2=4.65, p=0.03, df=-1, Nagelkerke pseudo R^2=0.06) and erosions (Chi^2=7.46, p=0.006, df=-1, Nagelkerke pseudo R^2=0.09) even when age was taken into account.

Conclusions: Occupation was expected to have an impact on bone mineral density and many of the individuals in this study undertook heavy manual labour. The fact that this showed no effect may be due to socio-economic factors, such as poor nutrition. It is possible that rotator cuff entheseal changes, particularly erosions may be impacted by localised low bone mineral density not captured by this study. Further research is needed to study the relationship between osteoporosis and cross-sectional geometry, which may be a better indicator of loading than occupation.

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LBP4

Skeletal defects emerge surprisingly early in murine models of secondary dystroglycanopathy

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Objectives: Defective bone function in muscular dystrophy (MD) is attributed to either steroid treatment or considered a secondary consequence of diminished mechanical loading. Of the 18 genes linked to secondary dystroglycanopathy, a heterogenous subset of MD with defective alpha-dystroglycan glycosylation, mutations in glycostransferase FKRP (fukutin-related protein) are the most common. Defective alpha-dystroglycan glycosylation disrupts cell:extracellular matrix interactions, compromises muscle development culminating in progressive wasting and bone defects. Herein, we explore the effect of FKRP knockdown on bone architecture and whether alpha-dystroglycan has a role in endochondral growth.

Methods: We examined skeletal phenotype in two mouse FKRP-deficiency models before overt deficiency in muscle force generation emerges. We used $FKRP^{KD}$, with 60-80% FKRP knockdown that results in perinatal death, and $FKRP^{MD}$ mice expressing Sox1 promoter-driven cre-recombinase resulting in survival via successful FKRP restoration in brain but not skeletal muscle. Bone architecture was assessed in micro-CT scans of PO neonatal femurs of $FKRP^{KD}$ and 12 week-old $FKRP^{MD}$ males (WT and Cre- controls); entire tibial

cortical bone analysis was undertaken in FKRP^{MD}.

Results: We found deficient bone volume ($p<0.05$), trabecular number ($p<0.05$), trabecular pattern factor ($p<0.01$) and structural model index ($p<0.01$) in neonatal FKRP^{KD} mutants indicating that bone was already weaker than in WT. Analysis of 12 week-old FKRP^{MD} mutants revealed similar trabecular bone changes, with significantly reduced bone volume, tissue volume and structural model index (all $p<0.05$) compared with Cre-controls. These FKRP^{MD} mice also exhibited significant reduction in tissue mineral density ($p<0.05$) and in cortical thickness in many tibial diaphysis regions, where shape (ellipticity), indices of mechanical strength (Imax and predicted resistance to torsion) were also compromised (vs controls). Immunohistochemistry revealed reduced levels of glycosylated a-dystroglycan and its ligand, laminin in the growth plate of these FKRP^{MD} mutants, suggesting a role for a-dystroglycan in endochondral growth.

Conclusion: These findings show that bone mass and architecture are adversely affected in two FKRP deficiency models prior to emergence of overt muscle dysfunction, suggesting that alpha-dystroglycan may exert additional, direct roles in bone development and homeostasis at least in this subset of MD patients.

LBP5

Sex-dependent associations between objectively-assessed physical activity and bone mineral density in community-dwelling older adults

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To determine cross-sectional and prospective associations of accelerometer-determined physical activity (PA) and bone mineral density (BMD) in community-dwelling older adults.

Community-dwelling older adults (N=209; 53% female; mean ± SD age 64.5±7.2 years) had PA assessed by an ActiGraph GT1M accelerometer over seven-days at baseline. Mean steps/day, and minutes/day of sedentary behaviour, light and moderate/vigorous intensity PA (MVPA) were estimated using previously established thresholds. BMD was assessed by total hip, lumbar spine and whole-body by dual-energy X-ray absorptiometry scans at baseline and approximately 2.5 years later. Relationships between PA and BMD were assessed using sex-stratified multivariable linear regression models adjusting for age, smoking status and body weight.

Mean steps/day and MVPA were (mean ± SD; 7707.9±3113.3) and (32.9mins±25.3mins) respectively. At baseline, steps/day was negatively associated with BMD at the trochanter (β, *P*; -0.32; <0.01), femoral neck (-0.26; 0.02), total hip (-0.33; <0.01), arms (-0.25; 0.03), pelvis (0-.30; <0.01) and whole-body (-0.24; 0.04) in men. In women, steps/day (0.24; 0.02) and MVPA (0.21; 0.03) were positively associated with leg BMD. After adjustment for body weight, negative associations were no longer significant in men, but in women, weight-adjustment resulted in positive associations for steps/day with BMD at the trochanter (0.23; 0.02), total hip (0.21; 0.02), legs (0.33; <0.01), and whole-body (0.22; 0.02), while sedentary time was negatively associated with pelvic BMD (-0.20; 0.03). MVPA was also positively associated with BMD at the total hip (0.17; 0.04), legs (0.28; <0.01), and whole-body (0.23; 0.02). Longitudinal analyses revealed steps/day was positively associated with total hip BMD change (0.28; 0.02) in men, and with femoral neck BMD change (0.21; 0.05) in women, but these associations were no longer significant after adjusting for body weight.

In community-dwelling older adults, body weight mediates associations between accelerometer-determined PA and BMD. Positive cross-sectional associations for steps/day and MVPA, and negative associations for sedentary time, were observed in women only. These findings suggest that body compositional differences may influence mechanical loading benefits of PA in older adults, although greater PA may have temporal positive effects in women.

LBP6

Repurposing glutamate receptor antagonists for the prevention of post-traumatic osteoarthritis

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Objectives: Synovial fluid glutamate concentrations increase in arthritis, AMPA/kainate glutamate receptors (GluRs) localise to osteoarthritic knees and NBQX (AMPA/kainate antagonist) reduces swelling, gait abnormalities and joint destruction in inflammatory and post-traumatic osteoarthritis (PTOA) models. NBQX is not approved for human use, therefore we have sought to repurpose successful Phase-1 clinical trial AMPA/kainate GluR antagonists. We hypothesise that these drugs will have similar therapeutic effects to NBQX in an ACL-rupture PTOA model.

Methods: Synovial fluid was obtained from patients following ACL rupture or meniscal tear injuries. Due to confidentiality purposes, drugs are anonymised A-D. For ACL rupture, load (12N, ElectroForce® 3200, BOSE) was applied to right knees of anaesthetised 12-week-old C57Bl6 mice. A single intra-articular injection of drug or vehicle was administered (n=5) immediately following ACL rupture. Over 21 days, knee swelling and lameness was measured (days 0, 1, 2, 3, 7, 14, 21). On day 21, animals were culled and knees harvested.

Results: Synovial fluid glutamate concentrations were increased in ACL rupture and meniscal tear patients. By day 2, drug A had reduced knee swelling by ~50% to levels no longer significantly different to day 0 pre-ACL rupture measurements. Vehicle control knee swelling remained significantly higher until day 7 ($p<0.01$, general linear model (GLM)). Drug C also reduced knee swelling to day 0 levels by day 2, whereas drug D ($p<0.05$, GLM) and vehicle control ($p<0.001$, GLM) remained significantly increased until day 7, and drug B ($p<0.05$, GLM) until day 14. Lameness scores were reduced by all drugs, with significant reductions compared to vehicle control on day 3 by drug B ($p<0.001$, GLM), drug C ($P<0.05$, GLM) and drug D ($p<0.05$, GLM).

Conclusion: We show that AMPA/kainate GluR antagonists, approved for human use, are effective at relieving inflammation and pain in PTOA. Repurposing of these drugs offers a rapid route to treatment of human PTOA, often occurring within 4 years of discovery. This work was supported by an MRC Confidence in Concept grant.

LBP7

Investigation of 3D femoral bone mineral density in women with hip fracture, frailty-matched women without hip fracture, and their daughters

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Objectives: If a mother fractures her hip before the age of 80, this confers on her daughters more than double the risk of hip fracture (compared to their peers) from the age of 65 onwards[1]. Intriguingly this increased risk is independent of he daughter's femoral neck bone mineral density (BMD) score at age 65. Tabensky[2], however, showed that the premenopausal daughters mothers with hip fracture had unremarkable femoral BMD within the normal range. In the FEMCO study we studied 3D femoral BMD measurements in women with hip fracture and in their daughters, but in addition recruited a group of age-matched women without fracture and their birth daughters.

Methods: 20 women who had sustained fragility hip fractures were recruited, along with 25 of their daughters. Also recruited were 25 mothers admitted to hospital for reasons other than hip fracture, and 32 of their daughters. BMD of the femoral neck was measured using QCTPro (Mindways, Texas USA) from CT to give 3D measurements of cortical and trabecular bone density.

Results: Women with hip fracture had lower femoral neck BMD than age-matched women without. Mothers without hip fracture and their daughters had strongly correlated femoral neck BMD (R^2=0.31, p=0.0011) indicating strong BMD inheritance. It was only in hip fracture families that there was no correlation (R^2=0.060, p=0.24), irrespective of whether mothers were over 80 at the time of injury. Heights were very strongly correlated between mother and daughter in both hip fracture and non-fracture groups.

Conclusions: In our small sample, the heritability of hip fracture risk did not manifest as low 3D BMD in daughters of hip fracture mothers, but there was strong heritability of BMD in daughters of women without hip fracture. The aetiology of the BMD-intependent hip fracture risk in daughters is currently unexplained.

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LBP8

Temporal gene expression patterns between developing embryonic mouse lower limb bones and calvaria indicate co-operation of mineralisation-associated proteins and phosphatases during skeletal biomineralisation

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Skeletal biomineralisation in vertebrates is a complex, highly regulated process which generates the intricate hierarchical and composite structure of bone tissue. At the ultrastructural level, mineralisation of collagen fibres is thought to be directed by phosphatase activity and non-collagenous proteins (NCPs) secreted into the extracellular matrix, many of which fall into the SIBLING family. While much work has focussed on the capacity of individual proteins in this process, little is known regarding the co-operation of NCPs and phosphatases in regulating mineral transport, nucleation and growth within the collagenous matrix.

RT-PCR was performed on RNA extracted from embryonic mouse lower limbs and calvaria (E14-E17). Gene targets included *Spp1, Ibsp, Mepe, Dmp1, Sparc, Phospho1* and *Alpl*. Skeletal development was visualised through Alizarin Red staining with optical projection tomography. Cell and tissue architecture was examined using histology with IHC for protein localisation of the phosphatases.

Mineralisation was observed to begin macroscopically at E15 in both long bones and calvaria. Complex patterns of temporal gene expression were observed at both anatomical sites with several genes expressed at E14 before mineral formation. Patterns varied over time with several targets exhibiting similar distributions, possibly indicating separate functions and co-operation between proteins at specific time points. Expression patterns were markedly different between sites, implying distinct mineralisation mechanisms between endochondral and intramembranous ossification modes. IHC for PHOSPHO1 and alkaline phosphatase showed divergent organisation of the phosphatases in the tissue architecture which was consistent at both anatomical sites. Given disparate gene expression patterns of these targets between sites, these data may indicate active control of gene expression linked to explicit protein functions at different time points to achieve controlled mineralisaton of collagen fibrils. Future work will include localisation of gene expression using fluorescent in-situ hybridisation (FISH) and examination of the ultrastructure of the mineralised collagen fibirils at each time point using transmission electron microscopy (TEM).

LBP11

New method for quantitative polarised light microscopy of laser-ablation machined sections of bones and joints

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Objectives: We have recently prepared very thin sections from the front face of bone blocks embedded in PMMA (which had previously been studied by backscattered electron scanning electron microscopy and x-ray microtomography) by the new technique of laser-ablation machining. These sections justify the development and use of new 3D high resolution light microscopic methods.

In conventional polarised light microscopy (PLM), positively birefringent crystals such as hydroxyapatite and/or negatively birefringent arrays of oriented molecules such as collagen appear brightest if they lie both in the plane of the section and at 45º to the axes of the crossed polarising filter elements. Birefringent elements appear black if they lie parallel to either polariser or analyser (or perpendicular to the plane of section). This situation prevents us from seeing the whole scene at once, because nothing can be seen in the dark sectors of the 'Maltese cross'.

Methods: We have overcome this problem by combining three grey-level PLM images. Digital images are recorded using green light with the polariser and analysor rotated 30º between each and used as red, green and blue components in a composite image. The colour maps the in-plane direction of the oriented molecular arrays irrespective of whether they are too small to be resolved. The intensity of the colour indicates the 'strike' of the molecules, i.e., the angle that they make to the plane of the section, brightest being parallel. An interpretive diagram has been developed which shows the colours for different orientations.

This method has been applied to an array of large and small bone and joint samples.

Results & Conclusion: The novel images refine understanding of bone, cartilage, calcified cartilage, Sharpey fibre bone, ligament, calcified ligament, tendon, calcified tendon and fibrous periosteum structure in bones.

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