BRS 2016 – Submitted abstracts

CC1

Atypical femoral fracture in a patient with Paget's disease

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Background

Long term treatment with bisphosphonates is associated with an increased risk of atypical femoral fractures (AFFs). This is a case of a 74 year old lady with Paget's disease who presented with AFF after 4 years since stopping bisphosphonates.

Presenting problem

A 74 year old lady with Paget's disease affecting the right tibia, presented with an 8 month history of pain in the left upper thigh on weight-bearing. She also had a 2 year history of pain in the right thigh and lower leg, after about 100 yards walking, attributed to confirmed spinal canal stenosis. She had undergone an intramedullary nail fixation of the right tibia in her 40-ies and a total knee replacement for secondary osteoarthritis at the age of 62. Parenteral bisphosphonates had been administered intermittently for 30 years until the age of 70. An isotope bone scan at the time showed an improvement in Pagetic disease activity. Two years later, when she developed right lower limb symptoms an isotope bone scan showed no significant change in the Pagetic disease activity and no new lesions. At the age of 73 elevated kappa-lambda light chains were noted and a skeletal X-ray survey revealed a focal area of cortical thickening in the left lateral mid-femoral diaphysis. A CT confirmed an incomplete left AFF and a mature periosteal new bone formation reaction in the lateral cortex of the right femur. Bone turnover markers (alkaline phosphatase and CTX) had been within the normal range since the age of 66, P1NP was low at 19 ug/L and 25(OH)D was 65 nmol/L at the time of pending AFF presentation. The incomplete AFF was treated with an intramedullary nail fixation.

Discussion

Bisphosphonates are the treatment of choice for symptomatic and active Paget's disease. However, long term use is associated with a higher risk of AFF, which diminishes after treatment discontinuation. Our patient with Paget's disease developed atypical stress reactions in unaffected femora with pending AFF four years after the last zoledronate infusion, which demonstrates the on-going (albeit diminishing) risk and serves as a reminder for clinicians to be alert to this rare complication of bisphosphonate treatment.

CC2

The treatment of Camurati-Englemann disease with losartan; a single case report

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Background: Camurati-Engelmann disease (CED) is a rare bone dysplasia characterised by hyperostosis and sclerosis of the diaphyses of the long bones and skull. It is caused by a number of autosomal dominant mutations that increase activity of transforming growth factor β1 (TGF-β1). It typically presents in mid childhood with bone pain, myopathy and progressive immobility. Evidence for treatment is based on a number of case reports, most of which describe the response to glucocorticoids. Losartan, an angiotensin-II receptor antagonist, is known to reduce expression of TGF-β1 and there are reports of two individuals with CED who showed significant improvement in pain and mobility in response to this treatment. We report a child with CED treated with losartan.

Methods: A 10 year old child with a clinical and radiological diagnosis of CED was commenced on losartan alone. The response to treatment assessed pain, function and mobility using the using the 6 minute walk test, Child Health Assessment Questionnaire (CHAQ) and formal assessment of gait. Bone health was assessed using biochemical markers of inflammation and bone turnover and radiological appearance including radiographs and densitometry. Anthropometric changes were also recorded. We observed for side effects, including specific monitoring for hypotension and electrolyte abnormalities.

Results: Over a period of 3 months, his 6 minute walk distance improved from 372 to 468 metres. On a scale of 0-3 both his global CHAQ score, which reduced from 1.5 to 0.9 and his pain assessment which fell from 0.6 to 0.2 showed improvement. No side effects were observed or reported.

Conclusions: The early response to treatment with losartan in our patient has led to improvement in pain and mobility; further outcomes will be evaluated in due course.

CC3

Atypical femoral fracture; single centre 10 year experience

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Objectives

Atypical femoral fractures (AFF) may complicate prolonged suppression of bone resorption, typically with oral/parenteral bisphosphonates. Prolonged suppression of bone resorption may promote micro-architectural damage and reduce fatigue life. Diagnostic criteria have been proposed (1). We describe clinical and radiological features of suspected AFF, within a single supra-regional centre

Methods

Review of suspected atypical femoral fractures identified between 2006-2016 within the metabolic bone unit, medical and treatment history, and fracture management

Results

We identified 71 (8 male) definite (n-66) or possible (n=5) adult cases of AFF, of mean (range) age 68 (39-91) years, most (n=63) associated with oral (alendronate, risedronate, ibandronate, etidronate) or parenteral (pamidronate, zoledronate) bisphosphonates for 8.9(2-21) years. Treatment indications were mostly osteoporosis but some had Paget's disease (n=1), osteogenesis imperfecta (n=3), bone marrow oedema syndrome (n=1), lumbago (n=1), and osteitis (n=1). 12 patients had significant steroid exposure. First AFF was as often left as right sided, occurred anywhere between the lesser trochanter and supracondylar flare, and were bilateral (with high degrees of symmetry) in 30 patients. AFF patients without prior bisphosphonate exposure trended to higher bone mineral density. Complete fractures were nailed and incomplete fractures, either nailed prophylactically, treated with teriparatide or observed. Where AFF was diagnosed prospectively the bisphosphonate was withdrawn. Some patients showed longitudinal evolution from periosteal elevation to full fracture. Subsequent choice of anti-osteoporosis treatment was problematic.

Conclusion

AFF is uncommon and sometimes misdiagnosed. Femoral fractures should be reviewed carefully for radiologic features of AFF, bilateral fracture must always be considered, and a high degree of suspicion is required among patients on potent anti-resorptive therapies. Our data also suggest that AFF may occur in patients with normal bone mass without relevant drug exposure. We also confirm in a few cases slow progression from early periosteal reaction to complete fracture, often accompanied by prodromal symptoms. The high degree symmetry in bilateral AFF suggests strong undefined mechanical determinants of fracture initiation and propagation

1. Atypical sub-trochanteric and diaphyseal femoral fractures: second report of a task force of the American Society for Bone and Mineral Research: AFF task force report. J Bone Miner Res 2014; 29;1–24

CC4

A case of stiff muscles

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A 46 year old male was seen in the rheumatology department after being referred by his GP with progressive pain and stiffness of the hands and lower limbs for the previous few months. His leg muscle felt tight and his hands were very stiff and he had a tendency to drop things. He had a past medical history of bronchiectasis, coeliac disease and hypocalcaemia of which the latter had been diagnosed in childhood. He was currently taking vitamin D 1000 IU daily. Both his father and son had hypocalcaemia, but his sister and two daughters were unaffected. On examination he had a slow stiff gait and walked with use of one stick. There was no joint swelling but his right index PIP joint was tender. He had difficulty in opening and closing his hands and flexing his knees; this appeared to be due to increased muscle tone as opposed to any inherent joint problem. Reflexes were generally brisk but there was no clonus. Investigations revealed a low calcium (2.10 nmol/l), normal CK, magnesium and plasma viscosity, and a low 25 hydroxyvitamin D3 (29 nmol/l). A clinical diagnosis was made of autosomal dominant hypocalcaemia (ADH) complicated by muscle spasms presumed secondary to albeit mild hypocalcaemia.

Genetic testing was subsequently performed to identify the basis for his ADH. No pathogenic variant was detected in *GNA11*, but in *CaSR* he had a heterozygous c.2497G>T substitution, p.Val833Phe. This change has not previously been reported either in the general population, or in other cases of ADH. p.Val833 is highly conserved across species, and lies within the functionally important extracellular loop 3 domain which has previously been reported to harbour ADH causative mutations. p.Val833Phe is predicted to insert a bulky aromatic ring into the structural region. Further analyes are planned to confirm this variant is responsible for ADH in this individual, by genotyping further family members.

In summary, a case of ADH is presented with somewhat atypical features characterised by muscle stiffness despite mild hypocalcaemia, caused by a novel *CaSR* mutation.

LB1

Evidence that genetic variants within a regulatory region of chromosome 1p13 predispose to Paget's disease of bone by regulating production of macrophage-colony stimulating factor

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Objectives: Paget's disease of bone (PDB) is a common skeletal disorder with a strong genetic component characterised by focal increases in bone turnover. We previously identified a susceptibility locus for PDB on chromosome 1p13, tagged by the single nucleotide polymorphism (SNP), rs484959, 87Kb upstream of the colony-stimulating factor 1 (CSF1) gene. This gene encodes macrophage colony stimulating factor (M-CSF) which is a strong positional candidate for PDB because of its role in osteoclast formation and survival.

Methods: Here we report upon fine mapping of the susceptibility region coupled with analysis of circulating M-CSF (Quantikine ELISA, R&D Systems, Abingdon, UK) concentrations in PDB patients.

Results: Two of the three top hits on GWAS were located within a regulatory region identified by histone acetylation (H3K27Ac) marks and DNaseI hypersensitivity sites. Sequencing of 272 cases identified three variants in linkage disequilibrium (LD) with each other that were clustered within a 1.4Kb region in proximity to transcription factor binding sites for AP1 and SRF. These variants were significantly overrepresented in PDB cases as compared with controls from the 1000 genomes project [rs72705287 (P = 8.12E-16), rs12123965 (P = 2.61E-15) and rs484959 (P = 1.93E-07)]. No coding variants were identified in CSF1 that could account for the association observed. Serum concentrations of M-CSF in a cohort of 51 previously untreated PDB patients were substantially increased (mean ± SD: 979±631 pg/mL) compared with the reference range (108 - 474 pg/mL) and fell significantly after bisphosphonate treatment to 703±380 pg/mL (p=0.001). Serum levels of M-CSF were higher in carriers of the PDB risk allele at rs484959 when compared with homozygotes for the protective allele (1010± 636 vs 818±392 pg/mL, p=0.12), suggesting that the predisposing variants may regulate CSF1 expression.

Discussion: These observations are consistent with the hypothesis that the chromosome 1p13 variants most probably predispose to PDB by upregulating expression of the CSF1 gene and also show that untreated PDB is associated with substantially increased M-CSF levels which are reduced by bisphosphonate treatment. Further studies are in progress using CRISPR/Cas to disrupt the region and investigate the mechanisms by which these variants regulate M-CSF levels.

OC1

Does PHOSPHO1 modulate bone's response to intermittent PTH?

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Intermittent administration of PTH(1-34) is currently the only anabolic therapy for osteoporosis. Recent, transcriptome sequencing analysis revealed that *Phospho1, a* bone-specific phosphatase essential for the initiation of mineralisation, is regulated by parathyroid hormone (PTH) in osteocytes (St John et al. 2015). This study therefore sought to determine whether the anabolic effects of intermittent rhPTH(1-34) may be attributed to the regulation of *Phospho1* expression.

In vitro, murine calvarial osteoblasts and human subchondral bone cells displayed increased expression of *Phospho1* in response to a 24-hour exposure to 50nM PTH(1-34) (6.9-fold and 5.7-fold respectively;P<0.001). *In vivo* studies initially investigated the effects of 6-hour exposure to rhPTH(1-34)(80µg/kg) on gene expression within the femur of 4-week-old wild-type male mice. This short exposure induced a significant increase in *Phospho1* (1.61-fold;P<0.01), and other mineralisation related genes, *Alpl* (1.29-fold;P<0.05) and *Smpd3* (1.45-fold;P<0.001) mRNA expression; expression levels of the transcription factors Runx2 and Trps1, similarly increased (P<0.05). To explore the role of Phospho1 in PTH-mediated osteogenesis, the effects of 14-days intermittent rhPTH(1-34)(80µg/kg/day) exposure was assessed in male wild-type and *Phospho1*$^{-/-}$ mice. Increased trabecular thickness, assessed by µCT, in response to PTH was observed in both genotypes (P<0.05). RT-qPCR analysis of wild-type femora however, revealed a more pronounced increase (*cf*. 6h challenge) in *Phospho1* (3.1-fold;P<0.05), *Alpl* (3.4-fold;P<0.001) *Smpd3* (2.9-fold;P<0.01) *Runx2* (2.6-fold;P<0.05) and *Trps1* (2.4-fold;P<0.05) mRNA following this prolonged PTH exposure. Similar transcriptional changes were also evident following intermittent PTH treatment for 28-days, albeit to a lesser extent (*Phospho1*, 1.8-fold;P<0.05 and *Smpd3*, 1.6-fold;P<0.05). Intriguingly, *Alpl*, *Smpd3*, *Runx2* and *Trps1* mRNA expression, in contrast, remained unaltered in response to such PTH treatment in the bones of *Phospho1*$^{-/-}$ mice.

The upregulation we observed in essential mediators of skeletal mineralisation, namely *Phospho1, Alpl* and *Smpd3,* provides a novel mechanism through which increases in bone mineral density might be mediated in intermittent PTH therapy. Furthermore reduced expression of the *Pth1r* in both the kidney (P<0.05) and femur (P<0.05) of *Phospho1*$^{-/-}$ mice, linked with the lack of induction of key mineralisation effector genes during PTH exposure, may highlight additional roles for PHOSPHO1 in regulating PTH responsiveness *in vivo*.

OC2

Effect of investigational treatment abaloparatide for prevention of major osteoporotic fracture or any fracture is independent of baseline fracture probability.

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Objectives: Clinical data suggest that daily subcutaneous injections of the investigational drug abaloparatide (80mcg) for 18 months significantly decrease the risk of vertebral and non-vertebral fracture compared with placebo in postmenopausal women. The aim of this study was to determine the effect of abaloparatide versus baseline fracture risk, assessed using the FRAX tool.

Material and Methods: Baseline clinical risk factors (age, BMI, prior fracture, glucocorticoid use, rheumatoid arthritis, smoking and maternal history of hip fracture) were entered into country-specific FRAX models to calculate the 10-year probability of major osteoporotic fractures with or without inclusion of femoral neck BMD. The interaction between probability of a major osteoporotic fracture and treatment efficacy was examined by a Poisson regression.

Results: 821 women randomized to the placebo group and 824 women in abaloparatide were followed for up to 2 years. At baseline, the 10-year probability of major osteoporotic fractures (with BMD) ranged from 2.3-57.5%. Data suggest that treatment with abaloparatide was associated with a 69% decrease in major osteoporotic fracture (MOF) compared to placebo treatment (95%CI: 38, 85%). The risk of any clinical fracture (AF) decreased by 43%; (95%CI: 9, 64%). Hazard ratios for the effect of abaloparatide on the fracture outcome did not change significantly with increasing fracture probability ($p>0.30$ for MOF and $p=0.11$ for AF). Similar results were noted for the interaction when FRAX probability was computed without inclusion of BMD.

Conclusions: Clinical data suggest that the investigational drug, Abaloparatide, may significantly decrease the risk of major osteoporotic fracture and any clinical fracture in postmenopausal women, irrespective of baseline fracture probability.

OC3

Longitudinal changes in bone structure as assessed by peripheral quantitative computed tomography and relationships with muscle health in older men and women

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Objectives: Cross-sectional analyses have shown strong associations between muscle size and both bone geometry and strength. There is little data on the effect of muscle size on changes in bone structure over time. We investigated this using a well phenotyped cohort of older men and women.

Methods: We studied 194 men and 178 women from the Hertfordshire Cohort Study each of which underwent peripheral quantitative computed tomography (pQCT) of the radius (66%) and tibia (14%) in 2004-5 and then again in 2011-12. Percentage change per year was calculated for muscle cross-sectional area (CSA) and diaphyseal bone parameters (total area (Tt.Ar), cortical area (Ct.Ar), cortical density (Ct.BMD), and polar stress strain index (SSIp)). These were then transformed using the Fisher-Yates rank-based inverse normal transformation to create sex-specific z-scores. Relationships between muscle and bone parameters were assessed using linear regression.

Results: The mean(SD) 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. Tt.Ar increased with age and at a greater rate in men than women in the radius (median: men 1.53%/year, women 0.94%/year, $p<0.001$). In both the radius and tibia, Ct.Ar reduced more rapidly in women than men (radius median: men 0.17%/year, women 0.49%/year, $p<0.001$). Rates of muscle loss were similar in men and women (forearm: men 0.75%/year, women 0.71%/year $p=0.424$). In men, rate of loss of Ct.Ar was positively associated with rate of loss of muscle CSA (β(95%CI): radius 0.31(0.17,0.45) $p<0.001$; tibia 0.18(0.03,0.33), $p<0.05$). A similar trend was shown in women but did not reach significance. Baseline muscle CSA was not associated with the rate of change in Ct.Ar.

Conclusion: Changes in diaphyseal bone structure with age differ in men and women. In men, the rate of loss of Ct.Ar is associated with rate of loss of muscle CSA and not its baseline level. This suggests that interventions to maintain muscle mass may help to ameliorate the age-related deterioration in bone health.

OC4

Osteocyte-derived microvesicles (MVs) stimulate adipocyte and osteoclast differentiation *in vitro*: a role for MVs in cell-cell communication in bone?

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Osteocytes are derived from osteoblasts and regulate bone turnover by signalling to other cells, but the mechanisms involved are not fully understood. Some of the pathways may involve extracellular microvesicles (MVs) although there is little published on the production and function of MVs by bone cells. This study investigated the effects of osteoblast/osteocyte i) conditioned medium (CM), ii) MV-depleted CM (MVDCM) and iii) MVs on adipocyte and osteoclast differentiation *in vitro*.

Mouse IDG-SW3 CM was collected during osteoblast to osteocyte differentiation (3-41 days). CM was subjected to sequential centrifugation (1,200rpm/10min; 3,110rpm/10min; 100,000rpm/2hrs) to pellet MVs. MVs were characterised by (1) Transmission EM (TEM), (2) Nanoparticle Tracking Analysis (NTA) and (3) total protein concentration. For adipogenesis, 7F2 cells were cultured (3-6 days) in adipogenesis medium and stained with Oil Red O. For osteoclastogenesis, RAW cells were primed with RANKL (2 ng/ml, 3 days), prior to treatment (3 days) and stained for TRAP. Treatments in each differentiation assay were either CM, MVDCM following ultracentrifugation, or MVs (20-150 µg).

TEM revealed that osteocytes secreted particles resembling MVs. MV concentrations from NTA in CM during osteoblast/osteocyte differentiation were 1.48+e08 - 3.8+e08/ml, with a mean particle size of 194.29nm. Re-suspended MV preparations had protein concentrations of 250-400 µg/150 µl. Adipogenesis was increased (p<0.05) in the presence of i) CM (day 7-30) and ii) MVs (20 or 40 µg) from day 14 (p<0.05, 40 µg) or day 17 (p<0.05, 20 µg) onwards. However, adipogenesis was reduced (p<0.01) by MVDCM (days 7-21). MVs (100-150 µg) also increased (p<0.01) osteoclastogenesis by ~10 fold, whereas MVDCM did not have an effect.

Osteocytes secrete large numbers of protein-containing MVs. Both CM and MV preparations stimulated adipogenesis, but MVDCM had a reduced effect when compared to CM, indicating a role for these MVs in adipogenesis. The results also show that MVs stimulate osteoclastogenesis *in vitro*. This work supports the hypothesis that osteocyte-derived MVs play a role in cell-cell communication. This has important implications in the development of new treatments for bone disease.

OC5

How does hypoxia-inducible factor (HIF) regulate osteoclastogenesis and bone erosion?

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Pathological bone loss and hypoxia co-exist in rheumatoid arthritis, bone metastatic cancer, primary bone cancer and osteoporosis. The hypoxia-inducible transcription factor, HIF, is stabilised in these conditions, correlating with disease progression and poor prognosis. Pharmacological inhibition of HIF protects from bone loss in murine models of osteoporosis, tumour-induced osteolysis and rheumatoid arthritis.

We are investigating the role(s) HIF plays in osteoclast formation and function. We have previously shown that hypoxic enhancement of osteoclast-mediated bone resorption is HIF-1α-dependent. We now examine whether HIF also regulates osteoclast differentiation and consider specific roles for the HIF-regulating prolyl hydroxylase domain (PHD) enzymes.

$CD14^+$ human monocytes were differentiated into osteoclasts with M-CSF and RANKL. HIF-1α mRNA and protein, as well as HIF target genes (LDHA, Glut-1 (Western blot), PGK-1 (luciferase assay)), were induced from differentiation day 5. HIF-1α siRNA affected neither the number of osteoclasts formed (TRAP-positive cells containing ≥3 nuclei) nor final resorption activity (lacunar resorption of dentine). Neither did HIF induction with $CoCl_2$ enhance differentiation. Interestingly, 24 hours hypoxia (2% O_2) at any time after differentiation day 5 increased resorption up to 2-fold in the mature cells (lacunar resorption of dentine, TRAP activity assay) without affecting osteoclast number.

Bone marrow-derived osteoclasts were differentiated *ex vivo* from mice with constitutive knock-down of PHD1-3, and so stabilisation of HIF. $PHD2^{+/-}$ marrow formed the same number of osteoclasts as wild-type controls, but the mature osteoclasts were 4-fold more resorptive (CTXI ELISA, TRAP expression). $PHD3^{-/-}$ marrow exhibited an accelerated rate of osteoclast formation. No phenotype was evident in $PHD1^{-/-}$ cells. Increased resorption by $PHD2^{+/-}$ osteoclasts was associated with over-expression of ANGPTL4 and altered mitochondrial metabolism, pathways which drive hypoxia-induced bone resorption in human osteoclasts.

This data suggests that effects of HIF on osteoclast bone resorption are predominantly mediated via PHD2, although small effects of HIF on osteoclast differentiation might be regulated by PHD3. This provides insight into how targeted manipulation of the HIF system could improve disease outcome in osteolytic bone loss conditions.

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OC6

Bone blood flow in diabetic mice and skeletal effects of Glucagon-like peptide-1 receptor agonists

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Type 2 diabetes mellitus is becoming an increasing epidemic and the disease predisposes to an increased risk of fractures and skeletal complications. One possible contributor to skeletal weakening in diabetes is a decline in blood supply. We tested the hypothesis that bone blood flow is impaired in diabetic mice and that chronic administration of Glucagon-Like Peptide-1 Receptor Agonists (GLP-1RA) can increase blood flow to bone, thereby stimulating bone formation and improving bone architecture.

Nine weeks old male diabetic (db/db) and control mice were daily injected subcutaneously for 28 days with saline or the GLP-1RA Exenatide (Ex-4) (10µg/kg/d) (n=10/group). The effect of Ex-4 on hind limb perfusion was measured by laser Doppler imaging. Tibial bone architecture was imaged by micro-CT ex-vivo and serum sclerostin levels measured by ELISA.

Diabetic mice had -40% lower bone blood flow than control mice ($P<0.0001$) at baseline. Ex-4 acutely increased tibial blood flow in diabetic mice from 15min of injection to a maximum of 25% increase compared to saline ($P<0.0001$). Similarly, blood flow was increased with Ex-4 in control mice but at a lower extend than in diabetic mice (+20%, $P<0.05$). No chronic effect of Ex-4 was shown when blood flow was monitored after the last injection. Diabetic mice have lower trabecular bone mass compared to controls, due to decreases in trabecular number and thickness. They also exhibit impaired bone connectivity, structure and cortical bone geometry. Ex-4 treatment increased trabecular bone volume (+49%, $P<0.01$), thickness (+8%, $P<0.01$) and number (+38%, $P<0.01$) in diabetic but not in control mice. Connectivity and structure were also improved as shown by decreased trabecular pattern factor (-29%, $P<0.0001$) and structure model index (-11%, $P<0.01$). Serum sclerostin levels were unchanged in diabetic mice compared to controls and increased in Ex-4 treated-diabetic mice (+26%, $P<0.001$) but not in Ex-4-treated control mice.

In conclusion, our results suggest that diabetic mice have lower blood flow and impaired skeletal structure and that Ex-4 exert a bone anabolic action in diabetic mice that could be in part due to an increased skeletal perfusion.

OC7

Do alterations in hip shape explain the increased risk of hip osteoarthritis in individuals with High Bone Mass?

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Objectives

Alterations in hip shape such as cam-type deformities are recognised risk factors for hip osteoarthritis. High Bone Mass(HBM) individuals are susceptible to a 'bone-forming' osteoarthritis phenotype. We aimed to determine whether hip shape varies in HBM, and its contribution to hip osteophytosis.

Methods

HBM cases, recruited by DXA database screening across 15 UK centres, had total hip or L1 BMD Z-score ≥+3.2; controls constituted unaffected relatives. AP pelvic X-rays were graded for osteoarthritis features and analysed using SHAPE; 58 points mark the proximal femur and acetabular eyebrow, Procrustes analysis adjusts for differences in hip size/location/rotation, before principal component(PC) analysis generates ten PCs (hip shape modes[HSM]); each describes and quantifies a linearly independent variation of hip shape. All HSMs were compared between HBM cases and controls, adjusting *a priori* for age, gender and height, using linear regression (standardised coefficients are presented).

Results

257 HBM cases (mean[SD] age 63.1[11.5]years, 75.9% female) and 131 controls (60.2[12.8]years, 48.1% female) were analysed. HSM3, HSM7 and HSM1 were mostly markedly different between HBM cases and controls; mean differences (β -0.29 [95%CI -0.52,-0.05],p=0.015), (-0.26 [-0.50,-0.03],p=0.029), (0.22 [0.00,0.43],p=0.047) respectively. Together these modes explained 44.7% of hip shape variation. In HBM, HSM3 represents larger femoral head size and reduced sphericity (cam-type deformity), HSM7 a more prominent greater trochanter, and HSM1 a larger greater trochanter, reduced femoral head sphericity and wider femoral shaft. Findings were robust to additional weight adjustment.

Hip osteophytes (grade≥2) were more common amongst HBM cases than controls (21.2% *vs.*12.0%; OR 1.98[1.04,3.77],p=0.03). Whilst HSM1 and HSM3 were independent of osteophytes, a 1SD increase in greater trochanter prominence(HSM7) was associated with OR 1.31 [1.05,1.64],p=0.02 of acetabular osteophytes.

Conclusion

HBM individuals have different hip shapes compared with controls, with greater cam-type deformity, larger trochanters and wider femoral shafts. Greater trochanter prominence is associated with osteophytosis in HBM, suggesting hip shape changes contribute to hip OA in HBM. Assuming biomechanical factors underlie these relationships, greater trochanter prominence may likewise represent a novel risk factor for hip OA in the wider population.

OC8

Hypomineralisation drives joint instability and osteoarthritis in mice

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Although often considered secondary, subchondral bone (SCB) thickening in osteoarthritic joints is one of the earliest detectable changes and is now considered a potential trigger for subsequent articular cartilage degeneration. PHOSPHO1, a bone specific phosphatase, is indispensable for bone mineralisation and its ablation in mice (*Phospho1*$^{-/-}$) alters bone material properties and decreases matrix mineralisation; changes that resemble the osteoarthritic SCB phenotype. Here we investigated whether PHOSPHO1 is involved in the aetiopathogenesis of osteoarthritic SCB sclerosis. Knee joints from 1-year-old male *Phospho1*$^{-/-}$ mice were examined for articular cartilage degeneration by OARSI grading and osteophyte formation by histological analysis. Gait analysis on 14-week old male *Phospho1*$^{-/-}$ and wild-type mice was performed using the CatWalk gait analysis system. Right knees of 16-week old male *Phospho1*$^{-/-}$ and wild-type mice were loaded three times/week for two weeks (11N) to examine, by micro-CT, the impact of mechanical loading on an inherently unstable hypomineralised joint. Aged *Phospho1*$^{-/-}$ mice showed increased average and maximum articular cartilage degradation scores when compared to the age-matched wild-type mice. Similarly, there was an increased incidence of osteophyte formation in *Phospho1*$^{-/-}$ joints. Gait analysis revealed significant reductions ($P<0.001$) in paw area and maximum contact area in all four limbs in *Phospho1*$^{-/-}$ in comparison to age-matched wild-type mice, suggestive that the *Phospho1*$^{-/-}$ mice are not putting their entire paw down during locomotion. In vivo joint loading increased SCB plate thickness in the lateral and medial femoral condyles in all mice. The extent of this load-induced increase was, however, significantly diminished in the *Phospho1*$^{-/-}$ mice. This deficit in load-induced osteogenic response in the *Phospho1*$^{-/-}$ mice was also apparent in the femoral epiphyseal trabecular compartment, where *Phospho1*$^{-/-}$ mice showed significant blunting of: i) load-induced increases in medial trabecular % BV/TV ($P<0.05$), ii) increases lateral trabecular pattern factor ($P<0.05$) and iii) increases in both the medial and lateral SMI ($P<0.05$). These data indicate that the hypomineralised bone phenotype in mice deficient in *Phospho1* provokes osteoarthritis pathology. Together these data suggest that mechanoadaptive changes in bone remodelling, due to local modifications in bone matrix mineralisation, underpin SCB sclerosis in osteoarthritis.

OC9

Pharmacological activation of autophagy by the polyamine Spermidine protects against osteoarthritis in vivo

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Ageing is universally linked to musculoskeletal deterioration such as bone loss and osteoarthritis (OA), in all sexes and ethnic backgrounds. Mechanisms known to regulate human cellular ageing may therefore play a role in the development of age-related musculoskeletal diseases, and targeting such mechanisms in OA may improve chondrocyte biology and protect against cartilage degradation. Autophagy is a well-conserved cellular process of waste removal and recycling. Defective autophagy is strongly associated with pathologies such as neurodegeneration, bone loss and OA. Spermidine is an endogenous polyamine also found in high quantities in aged cheese, whole grains, soy products, and has been shown to activate autophagy in a variety of cell types. Using advanced cellular and molecular studies and a surgical in vivo model of experimental osteoarthritis, we have explored the hypothesis that spermidine treatment will increase autophagy in chondrocytes, and may prevent against the development of arthritic disease.

Aged (12 months) vs young (2 months) mice showed a significant decrease ($p<0.01$) in the key autophagy maker LC3 in articular cartilage surfaces of the knee joints. Avulsed cartilaginous hips treated with spermidine showed increased LC3 I to II protein conversion compared to control (40% increase; western blot analysis) indicating increased autophagy compared to vehicle control. Experimental OA can be induced in mice following trasectionof the medial meniscus (destabilisation of the medial meniscus model (DMM)), after which characteristic features of OA develop such as cartilage degradation and increase in epiphyseal volume. After induction of DMM, mice were administered with either Spermidine (5mM) or water control for 8 weeks, and bone and joint assessed by uCT and histomorphometric analysis. Mice treated with spermidine showed improved OARSI disease score compared to control (10.00 ± 1.553 vs 4.846 ± 1.037; $p<0.01$), with increased bearing less tissue damage. MicroCT analysis revealed epiphyseal volume was unchanged in treated groups, compared to a significant thickening in control groups (3.759 ± 0.06906 vs 4.428 ± 0.2110; $p<0.01$).

This data suggests activation of autophagy by spermidine reduces tissue damage in an experimental OA model, and foods rich in spermidine may protect against the development of OA.

OC10

The cellular mechanosensory structure primary cilia, activate autophagy in chondrocytes and are dysregulated in arthritic cartilage

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Osteoarthritis is a debilitating ageing-related disorder affecting ~12% of the UK. Genetics and mechanical forces contribute to osteoarthritis pathogenesis, degraded articular cartilage and compromised repair. Chondrocytes are isolated cells which release matrix proteins to maintain cartilage integrity. Their sparse distribution implies robust self-preserving mechanisms operate to maintain optimal function. One such mechanism, Autophagy, involves the removal and recycling of defective proteins for continued protein production. Defective autophagy is linked to many age-related disorders (e.g. diabetes, neurodegeneration) where unwanted proteins accumulate to impair cellular function.

Primary cilia are single structures protruding from extracellular membranes of many cell types, and act as mechanosensors, sampling the external milieu and triggering appropriate cellular responses. However the mechanisms through which this occurs and their role in arthritic disease remain poorly described. We hypothesize that primary cilia expression is altered in OA and loss of these cellular mechanosensors impairs chondrocyte response to tissue damage.

Fresh articular cartilage samples were collected following knee replacement surgery under approved Ethical and HTA procedures. Each tibial plateau was portioned into worn/non-worn areas following gross examination. Following histomorphometric assessment, the cellular content of worn cartilage was surprisingly increased (61.67 ± 6.4Vs234.33 ± 27.5, $p<0.01$). Consequently, the expression of primary cilia detected in worn cartilage following staining for acetylated alpha-tubulin, was also increased x5 fold ($p<0.05$), as a possible response to degrading cartilage, increased mechanical strain. However, pharmacological inhibition of the ageing-related SirT1 enzyme (Ex-527, 500nM) known to be reduced in ageing cartilage, reduced cilia in healthy/worn chondrocytes (95.8 ± 2.2Vs78.49 ± 3.9, $p<0.01$). Induced in vitro mechanical strain (fluid flow (30 OPM)) in ATDC5 chondrocytes increased cilia expression and simultaneously, the rate of autophagic flux (assessed by LC3 I/LC3 II conversion). However, loss of primary cilia through deletion of the crucial intraflagellar transport protein 88 (ift88), prevented the strain-induced increase in autophagy.

Our data suggests that primary cilia activate autophagy following mechanical stimulation, and primary cilia expression may be altered in conditions where SirT1 is decreased, such as ageing cartilage, contributing to an impaired response to mechanical injury.

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OC11

Inhibition of BMP signalling reduces bone destruction in Multiple Myeloma

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Multiple myeloma is usually incurable. Even in states of remission, the bone marrow endosteal niche is reported to provide a protective microenvironment for quiescent, chemoresistant tumour cells. The mechanism by which this protection is achieved is unknown.

We addressed this *in vivo* by performing GSEA on RNASeq transcriptome profiles of sorted endosteal niche components from myeloma-bearing mice (and controls), and comparing tumour from central marrow and endosteal niche. Endosteal tumour, osteoblasts and mesenchymal stem cells (MSCs) from tumour-bearing mice showed positive enrichment for a bone remodelling gene set. One of the 4 most significantly upregulated shared genes in endosteal tumour and MSCs was BMP receptor *acvr1*, whereas in tumour-associated osteoblasts it was the most downregulated gene in this set.

The BMP signalling inhibitor LDN-193189 (LDN) has high affinity for ACVR1 and was used to assess the role of BMP signalling in myeloma *in vitro* and *in vivo*. In the myeloma-bearing KaLwRij/5TGM1 mouse model, it significantly improved trabecular ($p=0.05$) and cortical bone volume ($p=0.004$) and reduced serum TRAP levels ($p=0.003$). LDN had no effect on overall tumour burden, however altered the niche-preference (endosteal *vs* central marrow) of myeloma cells to favour the endosteal niche (ratio endosteal: central marrow myeloma distribution 0.23 vehicle group, 0.37 LDN group, $p=0.034$).

In vitro, tumour BMP signalling activity, measured by expression of BMP response genes *id1* and *smad6*, increased 10-fold when myeloma cell lines were cultured in contact with bone marrow MSC lines, but LDN significantly abrogated BMP signalling. Expression of *rankl* was significantly decreased by LDN in 2T3 cells and stromal cells from myeloma-bearing mice ($p=0.03$).

In summary, BMP inhibition improves myeloma bone disease, potentially by reducing RANKL levels thereby reducing osteoclast activity. Reduced osteoclast activity would allow preservation of the dormancy-favouring endosteal niche, and our results support this. We demonstrate the potential for inhibition of BMP signalling for the treatment of myeloma and the associated bone disease; it could also have use in prolongation of quiescent phases, e.g. MGUS and post-treatment remission.

OC12

Leukaemia inhibitory factor is increased in human bone-metastatic prostate cancer; a novel mediator of prostate cancer induced bone disease?

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Objectives
Bone metastasis is a frequent complication of advanced prostate cancer (PCa), giving rise to both osteolytic and osteoblastic lesions. Following tumour-bone metastasis, survival rates plummet and treatment options are limited. There is therefore an urgent need to elucidate the mechanisms that drive this metastasis to identify novel therapeutics and biomarkers which may better target this critical and fatal stage of disease progression. Leukaemia inhibitory factor (LIF) is an IL-6 family cytokine known to be involved in a wide range of biological functions including haematopoiesis and bone formation. The aim of this project is to determine the role of LIF in PCa-induced bone disease.

Methods
We have employed a powerful combination of in vitro and ex vivo techniques including; cytokine array analysis of patient samples, co-culture systems to mimic the tumour-bone microenvironment, protein arrays, qPCR and migration and invasion assays.

Results
Soluble LIF was found to be elevated in the serum of prostate cancer patients who subsequently developed bone metastases vs patients that did not metastasize (57.15% ± 17.06% increase, $p<0.01$). LIF mRNA and protein expression was increased in bone metastatic PCa cells, compared to non-metastatic cells (-0.6 fold, $p<0.001$), whereas LIF receptor expression was not associated with bone metastasis. Molecular knockdown of LIF had no effect on growth but dysregulated migration and invasion of bone metastatic PCa cells, supporting a functional role for LIF in progressive disease. PCa / stromal cell co-cultures demonstrate that PCa cells can upregulate LIF expression in stromal cells (4.5 fold, $p<0.001$), accompanied by an upregulation in receptor activator of nuclear factor kappa-B ligand (RANKL) (21 fold, $p<0.001$) and a downregulation of osteoprotegerin (OPG) gene expression (-0.83 fold, $p<0.0001$). In contrast, co-culture of PCa cells and stromal cells had no effect on LIF receptor expression.

Conclusion
Our results indicate LIF is strongly linked with bone metastases in human PCa patients, is a potential biomarker of disease progression and induces functional effects in cells of the tumour-bone niche to positively dysregulate RANKL/OPG regulation to favour osteolysis.

OC13

NBQX, an AMPA-kainate glutamate receptor antagonist, alleviates inflammation and pain related behaviour in two models of osteoarthritis

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Objectives: Synovial fluid glutamate concentrations increase in arthritis. Activation of kainate and AMPA glutamate receptors (GluRs) increase interleukin-6 release and cause pain. We previously found that AMPA and kainate GluRs localise to osteoarthritic bone, cartilage and synovium and that NBQX (AMPA/kainate antagonist) reduced knee swelling, gait abnormalities and joint destruction in a rat inflammatory-arthritis model. Here, we determined whether NBQX influenced inflammation and pain in two osteoarthritis models (medial-meniscal transection (MNX), non-invasive anterior-cruciate ligament rupture).

Methods: Rat right knees received MNX surgery (n=3 per group). NBQX (2.5mM, 12.5mM or 25mM) or vehicle control was injected intra-articularly into the right knee immediately after surgery and 7 days later. Over 21 days, knee swelling and hind-limb loading were measured (days 0, 1, 2, 3, 7, 8, 10, 14, 21). For ACL rupture, mouse right knees were loaded and the ACL ruptured (12N, 4Hz). NBQX (20mM) or vehicle control was injected (intra-articular) into the loaded knee immediately following ACL rupture (n=5). Over 21 days, knee swelling was measured (days 0, 1, 2, 3, 7, 16, 21). On day 21, all animals were culled and knees taken for histology.

Results: Vehicle control MNX rats had greater knee swelling compared to 25mM NBQX ($P<0.05$) on day 8, and 12.5mM ($P<0.05$) and 2.5mM ($P<0.01$) NBQX on day 14. Day 8 incapacitance readings showed vehicle control MNX rats had a greater weight bearing difference compared to 12.5mM ($P<0.05$), 2.5mM ($P<0.01$) and 25mM ($P<0.01$) NBQX rats. Day 21 inflammation and joint degradation was not reduced by NBQX treatment. In the ACL model, less knee swelling was found in NBQX mice compared to vehicle controls on days 7 ($P<0.05$) and 21 ($P<0.001$). From day 2, NBQX mice showed no difference in knee swelling compared to day 0, however, vehicle mice had significantly greater knee swelling compared to day 0. Day 21 degradation was reduced following NBQX treatment ($P<0.05$).

Conclusion: This study provides new evidence that NBQX treatment is effective at relieving inflammation, pain and joint degradation in osteoarthritis. Combined with our previous data from an inflammatory model, NBQX shows promise as a new disease-modifying drug for inflammatory and osteoarthritis.

OC14

Vascular smooth muscle cells and osteoblasts mineralise via distinct mechanisms

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Vascular calcification (VC) is defined as the deposition of hydroxyapatite in arteries and cardiac muscle. VC is associated with the transdifferentiation of vascular smooth muscle cells (VSMC) to an osteoblast-like phenotype. Our previous studies have shown ATP, UTP and synthetic ATP-analogues (α,β-meATP, β,γ-meATP, Bz-ATP) (≥1μM) act to potently inhibit both bone mineralisation and VC by ≤95%. Here, we compare the mechanisms by which extracellular nucleotides block these processes. Primary mouse VSMC and osteoblasts were cultured for up to 21 days in calcifying (2mM phosphate) and osteogenic (2mM β-glycerophosphate, 50μg/ml ascorbate) medium, respectively. Cells were treated with 1-100μM ATP, UTP, α,β-meATP, β,γ-meATP or Bz-ATP for the duration of the culture. We found basal alkaline phosphatase (TNAP) activity was 12-fold higher in mineralising osteoblasts compared to calcifying VSMC ($p<0.001$). Moreover, the TNAP substrate, β-glycerophosphate was ineffective in promoting VSMC calcification. The activity of NPPs, which generate pyrophosphate from ATP, was 10-fold higher in calcifying VSMCs ($p<0.001$). In differentiating and mature osteoblasts, extracellular nucleotides (≥10μM) inhibited TNAP activity by ≤50% ($p<0.001$). In contrast, ATP, UTP and ATP-analogues stimulated TNAP activity in calcifying VSMCs by ≤20-fold ($p<0.001$); at these concentrations VC was typically reduced by ≤90%. Prolonged treatment with ATP and related compounds (≤100μM) had no effect on osteoblast number and viability. However, in calcifying VSMC, cell number increased by ≤2.5-fold with extracellular nucleotide treatment; this was associated with a ≤70% ($p<0.001$) reduction in cell death. Since VC is also associated with increased apoptosis, extracellular nucleotides may prevent VC by promoting VSMC survival. These findings suggest that although the functional effects of extracellular nucleotides on bone mineralisation and VC are similar, the underlying cellular mechanisms are different. These results suggest that calcification in bone and vascular tissue involve quite distinct mechanisms. TNAP, which is expressed at low levels in VSMC, may not play a significant role in VC, in marked contrast to its critical requirement for bone mineralisation.

OP1

The pharmacological profile of a novel highly potent bisphosphonate, OX14 (1-fluoro-2-(imidazo-[1,2 alpha]pyridin-3-yl)ethyl-bisphosphonate), with reduced bone affinity, which is as effective as zoledronate in the treatment of myeloma bone disease in JJN3-NOD/SCID-γ mice.

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Bisphosphonates are used in the treatment of a variety of diseases with skeletal complications. With the development of more potent compounds, there is the potential for further improvement. One concept is to use compounds with a reduced affinity for bone, reducing their long-term retention and possible adverse events, as well as potentially enhancing their non-skeletal benefits. We hypothesise that a highly potent bisphosphonate with low bone affinity, known as OX14, will be as effective as bisphosphonates currently used in the clinic. The aim of this work was to evaluate the use of OX14 *in vitro* and *in vivo*. The binding of OX14 to hydroxyapatite and its ability to inhibit FPPS was compared to other bisphosphonates. The excretion rate and anti-resorptive potency of OX14 was assessed in a growing rat model. The effects of different doses of OX14 on bone integrity were assessed in naïve mice and its therapeutic effect was compared to ZOL in the JJN3-NSG murine model of myeloma. OX14 was more potent than ZOL at inhibiting FPPS and had a lower binding affinity to hydroxyapatite than ZOL, ALEN, IBAN or RIS. In a growing rat model, OX14 was more effective than RIS at increasing BMD. In addition, it was excreted into the urine to a greater extent than other bisphosphonates currently used clinically, indicating lower skeletal retention. In non-tumour mice, OX14 was shown to have a dose dependent effect on bone and was as effective as clinically relevant bisphosphonates. In a murine model of myeloma-induced bone disease, OX14 was shown to be as effective as ZOL at preventing the formation of osteolytic lesions. In summary, OX14 is a highly potent bisphosphonate with lower bone affinity than other bisphosphonates, and this may offer potential advantages in eventually treating patients who require bisphosphonates for their skeletal or non-skeletal benefits.

OP2

A fragility fracture increases the risk of incident cardiovascular events in men: Findings from UK Biobank

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Objective

The aim of this study was to investigate relationships between prior fracture and risk of incident cardiovascular events, in a population-based longitudinal setting.

Methods

UK Biobank is a large prospective cohort comprising 502,664 men and women aged 40-69 years, with detailed assessment at baseline. History of fracture was self-reported, and we obtained information on incident hospital admission (ICD-10) for ischaemic heart disease (IHD: I20-I25) and for any cardiovascular event (I63/I64 or I20-I25) through linkage to Hospital Episode Statistics (HES). We used Cox Proportional Hazards models to explore the longitudinal associations between past fracture and new hospital admission, in men and women separately, controlling for age, BMI, smoking, alcohol, educational level, physical activity, systolic blood pressure, calcium and vitamin D use, HRT (women) and additionally for heel bone ultrasound attenuation (BUA).

Results

Of the 502,664 individuals participating in UK Biobank, 19,981 were excluded because they lacked information on fracture history or BUA. This left 482,683 participants (median age 58 years; 54.6% women), 45,596 of whom reported a previous fracture (9.5%). In men, a history of any fracture was associated with increased risk of admission for IHD (adjusted HR: 1.18; 95%CI:1.03,1.36), but this was attenuated by inclusion of heel bone ultrasound in the model. The relationship between past fragility fracture (hip, spine, wrist, arm) and admission for IHD was stronger and remained significant with full adjustment (HR: 1.75; 95%CI:1.23,2.49). Associations with hospitalisation for any cardiovascular event in men followed a similar pattern [HR for admission after prior fragility fracture: 1.74; 95%CI: 1.24, 2.43). Although in crude models similarly increased risk of cardiovascular admission with prior fracture was noted in women, these became non-significant after adjustment ($p>0.3$).

Conclusions

Our findings from this large prospective population-based cohort demonstrate that prior fragility fracture is an independent risk factor for incident cardiovascular events. Further work may clarify whether this association is causal or represents shared risk factors, but these findings are likely to be of value in risk assessment of both osteoporosis and cardiovascular disease. This research has been conducted using the UK Biobank Resource.

OP3

Baseline osteoprotegerin antibody levels are associated with reduced spine bone density in coeliac disease, but not subsequent change in bone density.

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Objectives: Autoantibodies neutralising osteoprotegerin (OPG), a bone regulatory cytokine, have previously been demonstrated in a patient with coeliac disease. We aimed to establish whether these autoantibodies are associated with bone mineral density (BMD) in a coeliac disease cohort. Furthermore, we analysed whether these autoantibodies, and the use of bisphosphonates, affect hip and spine BMD Z-scores over time.

Methods: OPG autoantibodies were measured using a direct enzyme-linked immunosorbent assay in 250 coeliac patients. The autoantibody levels were categorised into high and low levels of autoantibody, with elevated levels defined as values greater than three standard deviations above the mean in 102 healthy controls. As a secondary outcome factors influencing the change in bone density over time were analysed in 102 and 100 participants who had undergone repeat spine and hip bone density scanning respectively.

Results: Using multivariate analysis, including age, bisphosphonate use, gender, height and weight as covariates, high levels of autoantibodies to OPG were significantly associated with lower spine BMD Z-scores, but not significantly associated with lower hip BMD Z-scores. No significant relationship between the autoantibodies and changes in BMD over time was observed. Those who were prescribed bisphosphonates were significantly more likely to have lower spine BMD Z-scores at their initial DEXA scan, but then showed significant improvement in spine but not hip BMD over time in multivariate analysis.

Conclusions: High levels of autoantibodies to OPG are significantly associated with lower spine BMD consistent with OPG antibodies having a pathological role in the development of osteoporosis in coeliac disease. We did not observe a fall in BMD over time in patients with OPG autoantibodies consistent with their being an epiphenomenon of osteoporosis, though it will be of interest to analyse changes in OPG antibody levels over time to see if this might be an alternative explanation. This study is the first, to our knowledge, to demonstrate benefit from the use of bisphosphonates in a large coeliac cohort, justifying standard treatments of osteoporosis in established coeliac patients.

OP4

Using the RUDY study platform to capture quality of life of adults with rare diseases of the bone.

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Objectives: Developing novel therapies for rare diseases is an important research priority. Although current methods involve recruitment from hospital clinics, this may only include the most severely affected individuals and so overestimate the population level burden of rare diseases.

Further, any new therapy will require an economic evaluation before implementation in the UK. However, there is a paucity of data on health-related quality of life measures in patients with rare diseases. We therefore compared quality of life across three rare bone diseases in adult using the EQ5D-5L.

Method: Adults with osteogenesis imperfecta (OI), fibrous dysplasia (FD) and X-linked hypophosphataemia (XLH) were recruited via the Rare and Undiagnosed Diseases Study (RUDY), a web-based platform for patient recuritment and assessment of patient reported outcomes including the EQ5D. The EQ5D-5L utility scores of OI participants were used to generate a cost-utility simulation.

Results: 82 adults completed the EQ5D-5L questionnaire. Overall there was a wide distribution of quality of life with moderate/severe problems commonly reported in the pain and discomfort dimension (OI 60%, FD 56%, XLH 65%). A cost-utility simulation showed that a hypothetical intervention which increased the health utility of the lowest utility tertile of OI patients to the mean utility level of the overall group over 10 years and costing £79,000 would be found cost-effective for the English NHS based on a £30,000 per QALY threshold.

Conclusion: These findings confirm that RUDY is recruiting patients across a range of quality of life with pain and discomfort domains most commonly affected. This is the first study to estimate the cost required to improve quality of life for adults with OI who have the lowest quality of life. A greater understanding of health-related quality of life amongst this population could help guide novel therapy developments and resource allocation.

OP5

Col2a1-driven TIMP3 disrupts endochondral growth and compromises bone structure and integrity: persistent transgene function in the chondrocyte-to-osteoblast lineage

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Development and growth of long bones are highly regulated, culminating in adult bone structure and function. During endochondral ossification, when bone is formed from a cartilage template, hypertrophic chondrocytes are thought to die by apoptosis, allowing blood vessel invasion and osteoprogenitor cells delivery. It was recently shown that these hypertrophic chondrocytes undergo trans-differentiation into osteoblasts. Herein, we use a transgenic approach, through the overexpression of Tissue Inihibitor of Metalloproteinases (TIMP)-3 in chondrocytes, to study this interaction and its consequences in adult bone.

A transgenic construct containing Col-2a1 proximal promoter region, first exon, first intron (gift from B. deCrombrugghe), was used to drive expression of human TIMP-3 and LacZ. Tibiae from 8week-old WT and TIMP3Tg/Tg mice were scanned by micro-computed tomography (Skyscan 1176, Belgium) and cortical and trabecular regions analysed. The femurs were used for three point bending, force deflection curves analysed to measure bending stiffness, yield and ultimate forces. Tibial bone surface strains were measured using digital image correlation. Strains in collagen fibrils and mineral of ulnae were measured. Mouse femur osteoblasts were cultured in vitro with/without osteogenic medium; cell number, alkaline phosphatase activity and osteogenic genes expression were calculated.

Overexpression of TIMP3 in cartilage resulted in shortened bones, linked to disruption in ossification centre formation. Their growth plates revealed additional expression of TIMP3 in the proliferating zones compared to WT, as well as significant width reduction in proliferating and hypertrophic zones. Despite a restoration in bone length at 8 weeks, tibial bone structure and mechanical properties remained compromised. Osteoblasts in culture showed no transgene expression but decreased differentiation capacities. Transgene LacZ expression was detected in a subset of bone cells in the cortices and trabecular regions of growing mice.

Our observations indicate that chondrocyte-selective overexpression of TIMP3 disrupts endochondral growth, particularly during early development, and produces persistent changes in skeletal structure which compromise the functional integrity of bone in post-natal and adult life. Our data also point to a persistence downstream impact of transient TIMP3 transgene expression which extends to influence osteoblast function within the chondrocyte-to-osteoblast lineage continuum to achieve these long-term changes in bone quantity and quality.

OP6

Ubiqutin-protein ligase UBR5 is a potent regulator of murine articular cartilage homeostasis and suppressor of osteoarthritis.

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Use of a novel conditional mouse model revealed the N-end Rule Ubiquitin-protein ligase UBR5 as a potent suppressor of osteoarthritis (OA). Based on our observations we hypothesise that UBR5 utilises Hedgehog signalling to govern articular cartilage (AC) homeostasis.

Animals conditionally homozygous for loss of Ubr5 function in the limbs (Prx1-Cre;$Ubr5^{fl/fl}$; aka Ubr5mt) displayed dramatic osteoarthritis-associated changes (n=8 for Prx-Cre control and Ubr5mt, Fishers exact p=0.002) to the AC that included: loss of superficial chondrocytes, increased numbers of hypertrophic-like chondrocytes, osteophytes and cartilage fibrillation. By 24 weeks of age, Ubr5mt animals exhibited extensive loss of AC down to the subchondral bone (n=6 for both control (Prx-Cre) and Ubr5mt genotypes, Fishers exact p=0.002). The AC loss was focal in nature and coincided with regions not covered by menisci, supporting a potential role for mechanical forces in mediating the physical degradation/detachment of Ubr5mt AC.

Supporting the histological observations, immunohistochemistry revealed dramatically altered expression patterns of markers of hypertrophic (Runx2, increased staining and number - 22% control Vs 86% Ubr5mt, Chi Sq p=<0.0001) and progenitor-like chondrocyte markers (Sox9, increased staining intensity and numbers - 33% control Vs 47% Ubr5mt, Chi Sq p=<0.001).

Based on results in *Drosophila*, we hypothesised the OA-associated changes were due to aberrant Hedgehog signalling. In agreement, Ubr5mt AC exhibited a dramatic increase in both canonical markers such as IHH, PTCH1 and GLI1 (e.g., GLI1: n=3, 15% control Vs 36% Ubr5mt positive cells, Chi Sq p=<0.0001) and the non-canonical-associated PKA activity (n=3, 21% control Vs 79% Ubr5mt positive cells, Chi Sq p=<0.0001). Treatment of Ubr5mt animals with Cyclopamine, an antagonist of canonical and agonist of non-canonical Hedgehog signalling, dramatically enhanced the Ubr5mt OA phenotype (n=4, Fishers exact p = 0.0286) as well as the number of PKA +ve cells (52% Vs 68%, n=3, Chi Sq p =<0.001).

In conclusion we believe that increased non-canonical, rather than increased canonical, Hedgehog signalling may be the underlying cause of Ubr5mt-associated OA. These findings highlight a potential therapeutic opportunity for OA treatment/prevention based upon the previously unknown relationship between UBR5 function, non-canonical Hedgehog signalling and articular cartilage homeostasis.

OP7

Ingestion of carbohydrate and protein immediately after an exhaustive run suppresses bone resorption and increases bone formation in trained male endurance runners.

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Objectives
To investigate the effect of feeding carbohydrate and protein (CHO+PRO) immediately or 2 hours after prolonged strenuous exercise, on the bone turnover response in endurance runners.

Methods
10 male participants (age 28±5 y, height 1.74±0.05 m, body mass 69.7±6.3 kg, VO_{2max} 63.0±5.0 $mL \cdot kgBM^{-1} \cdot min^{-1}$) completed this study. On three occasions participants performed a bout of treadmill running, at a speed equal to 75%VO_{2max}, until volitional exhaustion. Blood samples were collected before exercise and immediately, 1, 2, 3, 4 and 24 hours after exercise, for the determination of β-CTX, P1NP, PTH, PO_4, ACa and Ca^{2+}. The three randomised trials (Latin Square Design) consisted of; i) a placebo control (PLA) trial where a placebo solution was ingested immediately and 2 hours post-exercise, ii) an Immediate Feeding (IF) trial where the CHO+PRO solution (1.5 $g \cdot kgBM^{-1}$ dextrose and 0.5 $g \cdot kgBM^{-1}$ whey protein) was ingested immediately post-exercise and the placebo solution 2 hours post-exercise, iii) a Delayed Feeding (DF) trial where the placebo solution was ingested immediately post-exercise and the CHO+PRO solution 2 hours post-exercise. Data were analysed using repeated measures ANOVA and *post-hoc* Tukey's HSD. Area under the curve with respect to baseline was calculated from percentage change data (results not reported).

Results
At 1 and 2 hours post-exercise, β-CTX concentrations were significantly lower in the IF trial than in the DF and PLA trials ($P \leq 0.001$). At 3 hours post-exercise, β-CTX concentrations were significantly higher in the PLA trial than in the IF ($P \leq 0.001$) and DF trials ($P \leq 0.05$). At 4 hours post-exercise, β-CTX concentrations were significantly lower in the DF trial than the IF ($P \leq 0.005$) and PLA trials ($P \leq 0.001$). At 4 hours post-exercise, P1NP was significantly higher in the IF trial than the DF ($P \leq 0.05$) and PLA trials ($P \leq 0.005$). At 3 hours post-exercise, PTH was significantly higher in the IF than the DF trial ($P \leq 0.001$).

Conclusions
Following an exhaustive running bout, immediate ingestion of the CHO+PRO solution suppressed bone resorption by -22-61% from baseline and increased bone formation by +1-3% from baseline. Delayed ingestion of the CHO+PRO solution suppressed bone resorption by -44-65% from baseline but also suppressed bone formation by -10-11% from baseline.

OP8

Comparison of bone loss and muscle loss in ageing mice using iodine enhanced micro-computed tomography

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Osteoporosis and sarcopenia are major global healthcare problems affecting millions of elderly people. After the age of 60, reduced muscle mass and function may result in decreased mechanical loading and contribute to bone loss. Micro-computed tomography (micro-CT) is a technique widely used to measure bone mass and architecture in rodent models. Until recently it was challenging to analyse muscle and bone mass simultaneously. Recent advances in I_2KI enhanced micro-CT allow the analysis of muscle and bone mass in the same samples. This study evaluated trabecular bone properties and muscle size in the hind limbs of adult (6 months), n=8, and old (24 months) mice, n=7. The hind limbs were stained with a 3% I_2KI solution for 3 days. Samples were scanned using a Skyscan 1272 system (tube voltage 50kV, 200 µA and 0.5 mm Al filter) at a resolution of 4.5 µm for bone scans and 13.2 µm for muscle scans. On average, adult mice have significantly higher bone volume (20.85±0.76%) and trabecular number (3.49±0.14/mm) compared to old mice (7.52±0.23% and 1.38±0.04/mm respectively), p=0.001. As expected, trabecular separation was higher in old mice (0.32±0.01 mm) compared to adult mice (0.18±0.01 mm, p<0.001). Trabecular pattern factor was also higher in old mice (27.36±0.95/mm) compared to adult mice (17.75±1.68/mm, p=0.001) indicating a decrease in trabecular bone connectivity in the old mice. Moreover, the cross sectional area of tibialis anterior (TA), extensor digitorum longus (EDL) and gastrocnemius (GC) muscles at three different sites (distal, medial and proximal part of the tibia) was analysed. All three muscles were significantly smaller in old mice in the distal tibia, with decreases of: EDL 34%, GC 27% and TA 18% (p<0.05). In medial tibia, EDL and TA were significantly smaller in old mice (0.63±0.03 mm^2 and 3.77±0.03 mm^2, p<0.05) compared to adult mice (0.79±0.03 mm^2 and 4.49±0.11 mm^2). However, in the proximal area, there were no significant differences between adult and old mice in any of the 3 muscles measured. These data demonstrate that the rate of loss bone is much more substantial than that of muscle suggesting independent mechanisms of bone loss and muscle loss.

OP9

Does the adipokine, adiponectin, play a role in the coupling between fat and bone?

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Objectives
Clinical studies found that the circulating levels of adiponectin, a peptide hormone secreted from adipocytes, are inversely related to visceral fat mass and to bone mineral density (BMD) and it has been suggested that adiponectin plays a role in the coupling between fat and bone. Laboratory investigations of adiponectin activity in bone produced inconsistent results. The aim of our study was to elucidate the role of adiponectin in skeletal physiology through a comprehensive analysis of the bone phenotype of adiponectin-knockout (APN-KO) mice.
Methods
Ten wild-type C57BI/6J (WT) and 10 APN-KO (C57BI/6J background) female mice were culled at 8, 14, 21 and 28 weeks and groups of 12 mice at 37 weeks. BMD and body composition were determined longitudinally in the last two groups by DXA. Micro-architecture of femora was analysed by microCT (SkyScan 1172). Bone material properties were determined by nano-indentation and bone strength by three-point bending.
Results
The main differences between the groups were the lower cortical bone fraction in APN-KO at all the time points ($P<0.001$) and lower cortical thickness from week 14 onwards ($P<0.01$). Trabecular bone fraction was lower only in young animals ($P<0.05$). The longitudinal study found lower BMD in APN-KO mice ($P=0.04$) and a substantial reduction in percentage fat ($P<0.0001$). Bone material properties and strength were similar in the two groups. We found that adiponectin deficiency affects bone geometry and BMD negatively, but the differences in bone properties are fairly moderate and do not compromise bone strength.
Conclusion
Our study of the bone properties of adiponectin-knockout mice found negative effects of adiponectin-deficiency on bone geometry and density, suggesting a positive activity of adiponectin in bone. Assuming adiponectin has similar effects in humans, the low circulating levels of adiponectin associated with increased fat mass are therefore unlikely to contribute to the parallel increase in bone mass, and our results do not support a role for adiponectin in the coupling between fat and bone tissue.

OP10

The high bone volume phenotype of female nNOS KO mice is not maintained with ageing

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We have previously shown that female neuronal nitric oxide synthase knockout (nNOS KO) mice have increased trabecular bone volume. However, this study was performed in mice at 10 weeks of age only. To investigate whether the high bone volume is maintained during ageing, we compared 3-month- and 12-month-old wild type (WT) and nNOS KO mice using µCT. The tibias from 8 WT and 8 nNOS KO mice at each age were dissected, fixed for 24h in buffered formalin, stored in 70% ethanol, scanned using a Skyscan1272 µCT scanner at a resolution of 4.5 µm (50kV, 200 µA, 0.3° rotation step) and the proximal tibia analysed.
As before, the 3-month old nNOS KO mice had increased trabecular bone volume, with BV/TV increased by 35% ($p<0.01$), trabecular thickness by 11% ($p<0.05$), trabecular number by 20% ($p<0.01$), and connectivity density by 26% ($p<0.05$). In addition we found a change in the bone shape, with tissue volume increased by 17% ($p<0.01$) and periosteal circumference increased by 10% ($p<0.001$), indicating an enlargement of the bone and leading to an increase of the polar moment of inertia of 39% ($p<0.01$). At 12 months of age, the WT mice showed significant age-related bone loss, with a reduction in BV/TV and trabecular number of 63% ($p<0.0001$), a 79% increase in trabecular separation ($p<0.001$), and no change in trabecular thickness. At 12 months, there was no significant difference between WT and nNOS KO mice in BV/TV, trabecular number or trabecular separation. A significant increase of 20% was observed in the trabecular thickness of nNOS KO mice ($p<0.01$).
In conclusion, our results show that the high bone volume phenotype of female nNOS KO mice is not maintained during ageing As bone metabolism in nNOS KO mice is hypersensitive to changes in oestrogen levels, and oestrogen levels have been shown to be decreased in 12-month-old mice, the loss of the high bone mass phenotype may be due to an age-related decrease in oestrogen.

P001

Optimising the investigation and management of vitamin D deficiency at North Bristol NHS Trust

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Background: Vitamin D deficiency is common in the UK population, and is implicated in osteomalacia, cardiovascular disease, type 2 diabetes, and autoimmune conditions [1-3]. 'At risk' groups include older adults, the housebound, patients with chronic diseases (e.g. multiple sclerosis), patients taking antiepileptics, and black and ethnic minorities [4].

Methods: Our aim was to determine the level of understanding of doctors in North Bristol NHS Trust (NBT) regarding vitamin D deficiency. We issued an online questionnaire to all doctors working in the trust asking about in whom to measure vitamin D, what constitutes deficiency, how to treat it and how to follow up treatment.

Results: 52 clinicians responded to the survey; overall, knowledge of the thresholds for vitamin D deficiency and in whom to check levels was poor. Great variance existed in how respondents chose to treat vitamin D deficiency and follow up patients in the community. As a result, a multidisciplinary working group collaborated on developing a unified local guideline for treatment of vitamin D deficiency and insufficiency states, in line with National Osteoporosis Society recommendations [5,6]

Key Messages: Our survey demonstrated a distinct need for a Trust policy on investigating and managing vitamin D deficiency. New guidelines adopted by NBT should improve the way we approach vitamin D deficiency.

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P002

Predicting acute post-operative outcomes following hip fractures

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Objectives

Our main objective was to determine if pre-fracture morbidity can be used to predict the incidence of post-operative complications or the length of stay in the acute inpatient setting following a hip fracture. Our secondary objective was to determine if standard of care influences acute post-operative outcomes such as length of stay and post-operative complication rate.

Methods

We conducted a prospective observational study on a trauma ward in an urban teaching hospital to determine which factors are useful in predicting acute, in-patient post-operative outcomes.
Patient demographics and standard of care were collected via a chart based review and included age, medications, co morbidities, time to admission to ward, time to surgery and time in surgery.
Premorbid status was assessed via questionnaire based tools in an interview conducted within 48 hours of admission. Cognition was assessed using the Mini Mental State Exam (MMSE), frailty was determined using the SHARE-Frailty Index (SHARE-FI), nutrition was evaluated using the Mini nutritional Assessment (MNA) and co-morbidities were classified using the Charlson Comorbidity Index (CCI).

Results

60 patients were enrolled in this trial. Anaemia and lower respiratory tract infections were the most common acute post-operative complications seen.
Increased length of stay was associated with poorer cognition ($r=0.441$, $p=0.001$), frailty ($r=0.674$, $p<0.001$), malnutrition ($r=0.0.694$, $p<0.001$), increased number of comorbidities ($r=0.453$, $p<0.001$) and increased score on CCI ($r=0.532$, $p<0.001$).
Complication rate was influenced by cognition ($p=0.047$), frailty ($r=0.564$, $p=0.002$), grip strength in kilograms ($p=0.016$), nutritional status ($r=0.571$, $p<0.001$), CCI score ($r=0.588$, $p<0.001$), BMI ($p=0.001$) and number of medications ($r=0.293$, $p=0.023$).
Time to ward and time to surgery impacted on length of stay ($p=0.034$ and $p=0.039$ respectively).
Time in surgery showed a mild correlation with complication rate ($r=0.313$, $p=0.018$).

Conclusion

Acute post-operative complications and length of stay following hip fractures can be predicted based on pre fracture morbidity. Nutritional status, frailty and co-morbidities are the most predictive factors we assessed. We recommend assessing these factors in all hip fracture patients in order to flag those who are at a higher risk of developing post-operative complications or extended lengths of stay.

We, the authors have no known conflicts of interest to disclose.

P003 and P004 – abstracts withdrawn

P005

Generation of conditional knock out mouse for Alkaptonuria

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Alkaptonuria (AKU) is a disease caused by deficiency of the enzyme homogentisate 1,2-dioxygenase (HGD). Deficiency results in an increase in the circulating concentration of homogentisic acid (HGA), which over time is deposited as pigmented polymers in tissues including sclera, heart valves and cartilage, a process described as ochronosis. Joint ochronosis causes severe, early onset osteoarthritis.

A mouse model of AKU, Hgd^{aku} (MGI: 185664), carrying a single point mutation in the HGD gene after ENU mutagenesis was identified in 1994 following ENU treatment. However this mouse has other mutations that may influence the disease. Therefore a conditional Hgd-mutant mouse line enabling tissue-specific deletion of the *Hgd* gene would be advantageous. For this reason, we have obtained a mouse ES cell clone (EPD0642_4_D11) from the International Mouse Phenotype Consortium, which carries a ß-galactosidase reporter-tagged insertion. The strategy in this "knock out first" construct used to electroporate ES cells is to produce a knock out of the gene through the insertion of a cassette that disrupt transcriptional activity of the *Hgd* gene, which can be monitored by ß-galactosidase. Then using flippase, we will remove this cassette that includes the neomycin gene and restore the activity of the *Hgd* gene but leaving exon 4 flanked by two *LoxP* site ready for conditional excision.

Using these newly generated mice, we are identifying the *Hgd*-expressing cells during development and in adulthood in a temporal and spatial manner. The loxP sites permit conditional gene disruption by cre recombinase in a tissue-specific manner. Therefore, conditional deletion of the *Hgd* gene in liver will be achieved by mating the conditional *Hgd* knock out mouse with albumin cre recombinase mouse to evaluate whether the *Hgd* gene in the remaining cells and tissues can prevent the mutant mouse from establishing the disease.

P006

Aggrecan gene expression in chondrocytes is controlled by multiple regulatory elements

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Aggrecan (Acan) is a large aggregating proteoglycan which is functionally important for the mechanical properties of cartilage. It is required for the development and maintenance of mature cartilage. The loss of Acan is considered one of the first events in the development of osteoarthritis (OA). Little is understood about transcriptional control of Acan. This study aims to characterise cis-acting elements of Acan in skeletal elements.

We have used the publically available ENCODE consortium data to identify possible regulatory elements of Acan. The criteria for selection included: evolutionary conserved sequences, histone modifications such as mono-methylation of histone H3 lysine 4 (H3K4me1) and acetylation of histone H3 lysine 27 (H3K27ac). Nine possible cis-acting sequences were identified between -150kb to +60kb flanking the Acan gene, one of which was the known enhancer at -10kb by Han and Lefebvre, 2008. We cloned each of the sequences upstream of the HSP68 minimal promoter driving a LacZ reporter and generated transgenic mice. We found three upstream enhancer elements (-35kb, -65kb and -87kb) and one intronic region +26k that express in chondrocytes. The -65kb is active in hypertrophic regions in the limbs but is absent from the intervertebral disk. The -35kb and +26kb present in all chondrocytes at E15.5, irrespective of the stage of the chondrocyte. The final -87kb region shows weak expression during development in the limbs, ribs and skull; this progress in 8 week old mice as the expression becomes more robust in articular cartilage and the growth plate chondrocytes.

Electromobility Shift Assays (EMSAs) reveals that the transcription factor Sox9 interacts at multiple sites in all of the regions and mutations introduced into the sequences abrogates the binding. An inspection of the Sox9 binding sites mutations in vivo of the -35kb enhancer revealed a "shift" in the expression to areas other than the chondrocytes. We have explored the function and importance of these enhancers during development, adulthood and showed greater control of Acan gene than any other matrix genes studied up to date.

This work is supported by The Rosetrees Trust and the University of Liverpool

P007

Reduction of frontal plane knee moment caused by lateral trunk lean depends on step width

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The internal knee abduction moment (KAM) in osteoarthritis is reduced by increased lateral trunk lean (TL). Mechanistically, this occurs as the Centre of Mass (CoM) moves further over the stance leg. Since the size of the base of support constrains the CoM, an associated increase in step width (SW) would be expected in order to maintain stability. This study tested the effects of TL on SW and KAM in healthy participants (n=21) who performed normal and 6° TL walks. The latter was controlled via audio-visual biofeedback of the lateral trunk orientation. We found two distinct gait strategies in TL walk: widening the step width substantially (>50%) to permit an increase in the CoM displacement (WSW, n=12), or maintaining a baseline SW and minimally displacing the CoM by moving the hip/pelvic complex in the opposite direction (NSW, n=9). Whereas WSW doubled SW (0.12±0.03 v. 0.25±0.06 m, $F(1,12)=54.36$, $p<.0001$), NSW did not change SW, 0.13±0.03 v. 0.13±0.05 m, $F(1,7)=0.12$, $p=.743$. Both NSW and WSW reduced KAM impulse significantly in TL walk, 0.148±0.040 v. 0.131±0.046, $F(1,7)=5.56$, $p=.050$, and 0.195±0.038 v. 0.137±0.054, $F(1,12)=31.11$, $p=.0001$, respectively. However, these two distinct gait strategies resulted in unique patterns of KAM reduction across the stance phase. NSW reduced KAM impulse significantly in the initial half, 0.081±0.019 v. 0.060±0.019, $F(1,7)=26.57$, $p=.001$, but not in the later stance phase, 0.067±0.026 v. 0.072±0.035, $F(1,7)=0.49$, $p=.506$. WSW reduced KAM significantly in both, initial, 0.106±0.028 v. 0.077±0.037, $F(1,12)=16.73$, $p=.002$, and later stance phase, 0.088±0.017 v. 0.060±0.030, $F(1,12)=36.48$, $p<.001$. KAM peak results followed the pattern of impulse. These findings demonstrate that SW could play a significant role in the alterations of KAM profiles in lateral TL walk. This indicates that individualised gait re-training strategies could be adopted to target specific phases of knee loading. It also emphasises the need for careful consideration over the SW performance and control in future gait analysis studies.

P008

Is a lack of structural integration at the intervertebral disc-vertebra interface a factor in human lumbar spine degeneration?

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Collagen and the organisation of its bundles and fibres are hugely important to the mechanical and functional properties of musculoskeletal tissues. In the spine, the collagen within the intervertebral disc (IVD) has a highly organised hierarchical structure which has been well defined. However, how the tissue integrates with the cartilage hyaline endplate and vertebral endplate has not been well described in humans, despite failure in this area being implicated in the pathogenesis of disc disorders[1]. In this study we have used differential interference contrast (DIC) microscopy to examine the collagen organisation in this region.

Magnetic resonance images (MRIs) of cadaveric lumbar spines were graded using the Pfirrmann scoring system[2]. Motion segments from a range of degenerative grades (I–V) were cut sagittally. The right-hand side was fixed, decalcified and sectioned into three segments containing the anterior or posterior annulus fibrosus and the nucleus pulposus. Segments were cryo-sectioned (30µm) and fully hydrated sections visualised using standard light and DIC microscopy. The fibrous organisation at the IVD-vertebra junction was noted and lack of attachment assessed between the IVD and both cartilage and vertebral endplates and a percentage detachment calculated.

DIC microscopy revealed the fibrous organisation at the IVD-cartilage endplate interface with structural features such as bundles of collagen fibres continuing from the lamellae of the inner annulus fibrosus appearing to sub-divide within the cartilage endplate; similar bundles from the nucleus pulposus formed nodal insertions into the cartilage endplate centrally. Collagen fibres of the outer annular lamellae appeared to continue into the vertebral cortex. The structural integrity of the IVD and cartilage endplate was lost with increasing degeneration and detachment at the IVD-vertebra interface increase with more degenerate discs.

This preliminary work raises the possibility that a lack of attachment, especially at the disc-vertebra junction, may destabilise the spine and alter the mechanical environment of the cells in the IVD potentially contributing to the aetiopathogenesis of human IVD degeneration. Conversely, the microscopic structural features noted between the disc and the cartilage endplate may act to maintain attachment at this particular interface.

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[2]Pfirrmann, *Spine* 2001:26:1873-1878

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P009 – abstract withdrawn

P010

Small organic molecules in octacalcium phosphate

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Objective
Citrate plays an important structural role in bone mineral. Other small important acidic metabolites such as lactate and succinate also have high calcium affinity and may incorporate into bone mineral similarly to citrate. Different organic acid components in bone mineral are expected to cause differences in the physical properties such as solubility and toughness, and may contribute to bone disease. A synthetic citrate -- octacalcium phosphate composite provides a model of citrate incorporation into bone mineral. The objective of this study is to investigate the molecular structure of octacalcium phosphate (OCP) - small molecule composites as models for the inclusion of other organic acids in bone mineral, and their effects on its solubility.

Methods
Solid state nuclear magnetic resonance spectroscopy (ssNMR) was used to investigate the atomic structure of synthesised composites, supported by powder X-ray diffraction and scanning electron microscope (SEM). Density functional theory (DFT) was used to compute trial structures of OCP-succinate to compare with experimental results. The pH dependence of composite dissolution rates was compared using 31P solution state NMR.

Results
31P and 13C ssNMR spectra of several OCP-small molecule composites were characterized. The ssNMR technique of Rotational Echo DOuble Resonance (REDOR), and 2D through-space correlation experiments, yielded quantitative structural information on the mineral-organic interface, and on the spatial arrangement of mineral atoms, respectively. 43Ca ssNMR revealed the number of crystallographically distinct calcium sites in the composite structures.
Together, the data confirmed organic molecule incorporation into the mineral, with different organic molecules exerting different effects on the mineral lattice structure. 31P and 1H-31P heteronuclear correlation NMR spectra and SEM images of mixed organic acid-mineral composites strongly resembled those of bone mineral itself.
A high resolution computed structural model of OCP-succinate predicted the experimental results with reasonable agreement.

Conclusion
Many small organic metabolites can potentially incorporate into bone mineral in a similar manner to citrate. If this occurs, for instance in metabolic diseases, it will affect mineral molecular structure, morphology, and physical properties.

P011

Location, Location, Location: conserved differences in regional canine osteoblast behaviour

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Hip osteoarthritis is a cause of significant morbidity in people and their canine companions. Medical management is frequently insufficient, leading to surgery to relieve pain and regain mobility. Hip replacements are not without potential complications, including loosening and infection. Currently, there is a focus on uncemented implants to decrease these problems, however these rely on the biology of the femur for osseointegration and long-term stability. It has been shown in humans that osteoblasts from different types of bone from the same anatomical region have inherent programmed diversity in their growth and differentiation. Our main goal was to determine if similar bone type-related differences are observed in canine femoral samples.

Femoral heads from three canine hip replacement surgeries were collected. Fragments of bone from subchondral, trabecular and cortical areas of the femoral epiphysis were collected and washed, trypsin-digested and incubated in 0.2% collagenase. The fragments were seeded in DMEM+10% FCS at 37°C, 5% CO2, grown until confluence and the cells re-seeded (n=6) at a density of $1.3x10^4$ cells/cm^2. We determined cell proliferation after 4 days with crystal violet staining and basal alkaline phosphatase activity 24h post-confluence.

Osteoblasts from different skeletal locations showed no differences in cell proliferation or doubling time. In contrast, basal alkaline phosphatase activity was higher in trabecular than in subchondral ($p<0.05$) or cortical ($p<0.001$) bone osteoblasts. We are now optimising culture conditions to stimulate matrix mineralisation activity.

Our preliminary data indicates that canine osteoblast activity differs between trabecular, subchondral and cortical bone. This could have significant implications for future design of implants, leading to improved longevity and faster recovery from hip replacement surgery.

P012

Primary osteoblast culture from *Canis lupus familiaris* (domestic dog)

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Objective: Effects of bone organ size on isolated osteoblast behaviour are unknown. With two orders of magnitude range in body mass, dog breeds are well-suited to determine such relationships.

Methods: Femoral heads from three canine hip replacement surgeries were collected. Bone fragments were washed in PBS+AB/AM, trypsin-digested and incubated in 0.2% collagenase. Cells from resultant supernatant were seeded in DMEM supplemented with 10% FCS at 37°C, 5% CO_2, grown until confluence and then re-seeded (n=6) at a density of $1.3x10^4$ cells/cm^2. We determined cell proliferation after 4 days with crystal violet staining and basal alkaline phosphatase (TNAP) activity 1, 7 and 14 days post-confluence.

Results: Three specimens were collected, 2 females aged 1 and 9 years with 30 and 33kg respectively, and one male aged 6 years of 39kg. Median cell number reached $52.5x10^3$ [$30.1-72.5x10^3$] cells/cm^2 with doubling time of 3.5 [1.6-4.9] days. We found that cells from the 1 year-old female had greater cell count and shorter doubling time than the 9 year-old female ($p<0.001$). TNAP activity at day 1 post-confluence was increased in the older female sample when compared to the 1 year-old (0.01 [0.01-0.02] vs 0.03 [0.02-0.04] U/min/mg protein; $p<0.001$). This difference was not found at subsequent time-points since TNAP activity in the 9 year female sample decreased at day 7 post-confluence. TNAP is a key enzyme that has increased expression in mineralizing conditions and we are now optimising culture conditions to stimulate matrix mineralisation activity.

Conclusion: Age is an important factor in skeletal maturation/senescence: growth plates in dogs close at 12-18 months old and seniority is reached at 5-13 years depending on dog size and breed. These data provide an important step towards cell culture optimization for canine osteoblasts highlighting the importance of obtaining skeletally mature, but not geriatric, samples in comparative biology studies.

P013

Primary osteoblast culture from *Vulpes vulpes* (red fox)

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Objective: Effects of bone organ size on isolated osteoblast behaviours are unknown. Exhibiting a hundred-fold range in body mass, inbred canines are an ideal species to determine such relationships. We have therefore undertaken initial studies in both male and female red foxes (*Vulpes vulpes*), the most abundant and accessible wild canid member in the United Kingdom.

Methods: Femoral heads were removed from five fresh red fox cadavers and bone fragments washed in PBS+AB/AM, trypsin-digested and incubated in 0.2% collagenase. Cells from resultant supernatant were seeded in DMEM + 10% FCS + AB/AM at 37°C, 5% CO_2 , grown until confluence and then seeded (n=6) at a density of $1.3x10^4$ cells/cm^2. We determined cell proliferation after 4 days with crystal violet staining and basal alkaline phosphatase (TNAP) activity 24h post-confluence.

Results: Five foxes were collected; four females ranging from 4.6-4.9kg and one male weighing 5.9kg. Median cell number reached 35395 [25220-40106] cells/cm^2 with doubling time of 2.7 [1.9-3.0] days. The male sample had greater cell number 85184 [71789-88947] cells/cm^2 and shorter doubling time 1.9 [1.8-2.1] days when compared with female samples (34211 [15421-54000] cells/cm^2 and 2.9 [2.0-6.9] days; $p<0.0001$ for both parameters). Primary isolated cells (pre-osteoblasts) from foxes had at 24h post-confluence a median TNAP activity of 0.018 [0.011-0.023] U/min/mg protein. No differences were found among individuals.

Conclusion: As far as we are aware this is the first report of primary osteoblast culture from any fox species. TNAP is a key enzyme involved in mineralization and we are now optimising culture conditions to stimulate matrix mineralisation activity. These data provide a baseline from which bone scale at anatomical level can be related to isolated osteoblast behaviours in diverse canid species.

P014 – abstract withdrawn

P015

Micromechanical properties of articular cartilage and subchondral bone of the human knee joint

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Objectives: Finite element (FE) modelling has previously been used to explore the morphology and functionality of the human lower limbs, and represents the material properties of each structure modelled. As such, accurate representation of material properties obtained in vitro is essential for the realistic prediction of mechanical behaviour, thus aiding clinical investigations. This research aims to collate subject specific material properties of human knee joint articular cartilage and subchondral bone, using multiple regional samples from individual cadavers, in order to aid the construction of a more accurate FE model.

Methods: Preliminary tests incorporated two young healthy human cadaver knee joints (31 and 49 years old), which were dissected and eight articular cartilage and subchondral bone samples were harvested from the medial and lateral femoral condyle, and medial and lateral tibial plateau. Nanoindentation was used to calculate the elastic modulus, using a dynamic method employed with a 100µm diameter flat punch tip for the cartilage, and a quasi-static method employed with a Berkovich pyramidal tip (radius 20nm) for bone, incorporating ten spatially correlated indents per sample. Biologically realistic hydration status of soft tissue was maintained through partial submersion in phosphate buffered solution during testing.

Results: Material properties from the 31 year old cadaver presented with a mean global elastic modulus of 3.81MPa (localised range 1.43-6.61MPa) for cartilage, and 13.10GPa for subchondral bone (localised range 6.7-16.97GPa). Material properties from the 49 year old cadaver presented with a global elastic modulus of 1.90MPa (localised range 0.49-5.15MPa) for cartilage, and 13.50GPa for subchondral bone (localised range 12.84-14.54GPa). Articular cartilage showed high biological variability in both cadavers, whilst subchondral bone also showed high variability in one cadaver, across varying regions of the knee joint, therefore questioning the accuracy of previous FE model representations of such structures.

Conclusion: Typically, FE modelling represents biological material properties as a global structure with one suggestive value; however current research indicates a more local approach should be considered when constructing such models, in order to gain more biologically realistic behaviour of the human knee joint, in response to mechanical loading.

Acknowledgements: BBSRC and the School of Engineering, University of Liverpool.

P016

FGF-2 promotes osteocyte differentiation through increased E11 expression in vitro

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E11 is critical in the early stages of osteocytogenesis and its expression is induced by fibroblast growth factor 2 (FGF-2) released from cartilage upon injury both *in vitro* and i*n vivo*. Here we sought to determine whether FGF-2 regulates osteocytogenesis through increased E11 expression. MC3T3 clone-14 and primary osteoblast cells were exposed to different concentrations (0 - 50ng/ml) of recombinant murine FGF-2 for various time periods up to a maximum of 24hrs. Whole calvaria were subjected to similar FGF-2 challenge for 24hrs. *E11* and osteocyte/osteoblast marker mRNA was quantified using RT-qPCR and protein expression by western blotting. Immunofluorescence examined E11 expression and cell morphology. MC3T3 cells were treated with E11 siRNA for 24hrs and afterwards challenged with FGF-2 for 24hrs. FGF-2 exposure for both 4- and 24-hrs concentration-dependently increased *E11* mRNA expression ($P<0.05$) in MC3T3 cells and in primary osteoblasts. Similar increases were observed in primary calvaria organ cultures treated with FGF-2. Western blotting data confirmed the RT-qPCR data, revealing a concentration-dependent increase in E11 protein in FGF-2 treated cells. This increase in E11 expression is mediated by phosphorylation of the ERK intracellular pathway as shown by increased phosphorylation of p44/p42 in MC3T3 cells treated with FGF-2 for 15 minutes. These FGF-2 induced changes in E11 were also accompanied by significant ($P<0.01$) increases in *Phex* and *Dmp1* (osteocyte marker) expression and decreases in *Col1a1, Postn, Bglap* and *Alpl* (osteoblast markers) expression. E11 distribution in control cells was uniformly distributed within the cytoplasm and concentrated in the perinuclear region. This contrasted with MC3T2 cells treated with FGF-2 which promoted dendrite formation, and immunofluorescence microscopy revealed a modified E11 distribution that was concentrated in these dendrites. siRNA knock down of E11 achieved a 70% reduction of basal E11 mRNA expression and effectively abrogated FGF-2-related changes in E11 expression. Together, these data suggest that FGF-2 promotes osteocytogenesis through ERK mediated increased E11 expression.

P017

The response of fibroblast growth factor-23 (FGF-23) to teriparatide in postmenopausal osteoporosis.

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Objectives: FGF-23 is a phosphate regulating hormone and its production may be stimulated by circulating levels of 1, 25-dihydroxyvitamin D 1, 25-$(OH)_2$D. Teriparatide administration increases levels of 1, 25-(OH)2D, however it is unclear whether this mediates changes in FGF-23 levels. The aims were i) to determine the effect of teriparatide treatment on circulating levels of FGF-23 and 1,25-$(OH)_2$D and ii) to describe the time course of effect in postmenopausal women with osteoporosis.
Methods: Eighteen postmenopausal women (mean age 65.8 years) with osteoporosis, defined as a DXA BMD T-score of ≤-2.5 at the hip or lumbar spine, received teriparatide (Forsteo 20 mcg daily) subcutaneously for 2 years with daily elemental calcium (500 mg) and vitamin D supplementation. Fasting serum was collected at baseline then at weeks 1, 2, 4, 12, 26, 52, 78 and 104. The C-terminal FGF-23 was measured using an ELISA (Biomedica Gruppe) and 1, 25-$(OH)_2$D using an automated immunoassay (iSYS-IDS).
Results: At baseline, mean levels of FGF-23 and 1, 25-(OH)2D were 0.481 pmol/L (95% CI 0.381 to 0.607) and 60.7 pg/ml (95% CI 52.6 to 70.2) respectively. At week 1, levels increased significantly from baseline by 20.6% (95% CI 3.1 to 41.1) for FGF-23 and 86.9% (95% CI 55.1 to 125.2) for 1,25-$(OH)_2$D, $p<0.001$. The increase from baseline in both largely persisted over the 104 weeks of teriparatide treatment, though FGF-23 levels were not significantly different at the final time point.
Conclusion: Treatment with teriparatide was associated with early increases in both FGF-23 and 1, 25-$(OH)_2$D. The similar timescale of these changes suggests that the increase in FGF-23 may be mediated, at least in part, by the increase in 1, 25-$(OH)_2$D.

P018

Musculoskeletal and performance characteristics of exceptionally active older people: comparison between life-long runners, those taking up competitive running at a later age and a recreationally active control group.

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Regular physical exercise is known to be valuable in reducing the osteopenia associated with ageing. There are numerous schemes encouraging middle-aged and older people to take up regular exercise. However it is not known whether taking up exercise comparatively late in life confers the same benefits as life-long activity.

The present study examined the musculoskeletal characteristics of master athletes, all of whom had competed at a regional level of athletics in sprint, middle or long distance running within the past two years (n=67, mean age 70.5±0.74). These were compared with a group who had taken up regular competitive running after the age of 50 years but had no prior history of competing (n=106, mean age 68.6±0.54) and a third group of 'normally' active subjects (n=273, mean age 74.1±0.29).

All participants gave informed consent and completed whole-body dual-energy x-ray absorptiometry scans to determine body fat %, whole-body bone mineral density (WBMD), leg BMD and leg lean mass. Body fat % was 8.8% and 8.2% lower in life-long runners and later-life runners, respectively, compared with controls (both $p<0.0001$). Leg lean mass was similar for all groups. Compared with the non-athletic controls, people who had trained all of their lives had 4.2% higher WBMD and 8% higher leg BMD ($p<0.0005$), while those taking up exercise later in life had 4.2% higher WBMD and 7% higher leg BMD ($p<0.0005$) compared with the normal control group but with no difference between the two groups of athletic people for BMD measurements. There was no sex x training group interaction, indicating that the benefits seen in the athletic groups were similar for men and women

These results reveal that people who took up regular, running in middle age had similar BMD and body fat as those who had competed all of their adult lives, both were significantly better than values taken from recreationally active older people. There were, however, no apparent benefits for lean leg mass. Athletic training is associated with better skeletal health, but training throughout adult life gives no additional skeletal or performance advantage compared with taking up competitive running at a later age.

P019

Knees and Teeth Syndrome (KaTS) in cats - patella fractures in felines with persistent deciduous teeth

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Fractures of the patella were rarely reported in the feline until recently when it was recognised that cats would often suffer bilateral fractures and then subsequently go on to suffer atraumatic fractures of other bones. Intensive study of these cats has identified some common features. Patella fractures in cats are usually mid body transverse fractures. They can be seen in cats as young as four months of age, with a median age of 2. The onset is often acute although significant trauma has not necessarily preceeded the lameness. The condition is often bilateral with the second patella fracturing several months after the first (median gap of three months). Many affected cats have a history of retained or persistent deciduous teeth, a condition that is very rarely seen in the normal cat population. Male and female cats are affected and there is no breed predispostion.

The patella will usually appear sclerotic on radiographs prior to fracture. Subsequent fractures include humeral condylar fractures, proximal tibial fractures, bilateral acetabular or ischial fractures and femoral neck fractures. These subsequent fractures are all similar in type and location between the KaTS cats and they are not commonly seen in normal cats.

Genetic analysis, blood analysis and some preliminary biochemical analysis has been performed in these cats but an underlying cause has not been identified. Over 100 cats have been identified with a worldwide distribution. Some factors link it with osteogenesis imperfecta, but the cats have normal bone density radiographically, and sclerosis is a feature of some of the bones prior to fracture. The sclerosis would link it with a disease of increased bone production or failure to remodel bone. The retention of deciduous teeth is an interesting feature that potentially links it with dentinogenesis imperfecta but histological analysis of the teeth has not as yet shown any abnormalities. Work is ongoing to elucidate the cause of this condition, and comments and suggestions as to what to test for, and to know if there are similar conditions in the human, are welcomed.

P020

Weight is associated with hip shapes known to predispose to osteoarthritis: a cross-sectional study in peri-menopausal women

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Objectives
Hip shape is associated with fracture risk and a predisposition to osteoarthritis, yet determinants of hip shape are currently unclear. Mechanical loading is known to effect bone remodelling, hence body weight may influence hip shape. We conducted a large cross-sectional study to examine the association between weight and hip shape.

Methods
We obtained hip DXA scans for the mothers within the Avon Longitudinal Study of Parents and Children (ALSPAC) cohort. Hip images were analysed using SHAPE, which automatically places key geometric points around the femur and conducts principal component (PC) analysis to generate independent hip shape modes (HSM). Weight, lean mass and fat mass were regressed against each of the top ten HSMs adjusting for age and height (standardised coefficients are presented).

Results
4465 mothers with mean (SD) age 48 (4.5) years old and BMI 26.6 (5.31) kg/m2 were studied. 2677 (60%) of the participants were premenopausal, 1172 (26%) post-menopausal and 616 (14%) had no menstruation data. HSM 2 and 9 were most strongly associated with weight (β -0.22, 95%CI [-0.25,-0.19], p-value 1.5x10-46 and -0.15, [-0.18,-0.12], 2.1x10-23 respectively); together these explain 17% of hip shape variation. HSM2 includes variation in acetabular coverage (pincer-type deformity), femoral neck width and angle. HSM9 describes changes in femoral head size and sphericity (cam-type deformity). Both showed similar associations with lean mass: HSM2 (-0.21, [-0.24,-0.17], 4.7x10-33), HSM9 (-0.19, [-0.22,-0.16], 6.2x10-28) and fat mass: HSM2 (-0.20, [-0.23,-0.17], 8x10-41), HSM9 (-0.12, [-0.15, -0.09], 1.6x10-16).

Conclusion
In peri-menopausal women, weight is strongly associated with hip shapes resembling pincer-type and cam-type deformities, known to predispose to osteoarthritis. Furthermore, weight is associated with variation in femoral neck angle and width, which has previously been linked to fracture risk. The relationship between weight and hip shape appeared to be explained by equivalent associations with both fat and lean mass, consistent with weight acting predominantly via a biomechanical as opposed to metabolic mechanism.

P021

Withaferin A is a potent stimulator of chondrogenesis and longitudinal bone growth in rats

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Objective: We investigated the effect of Withaferin-A (WFA) from constituent herb *Withania somnifera* leaf in bone growth, achievement of peak bone mass and its chondro-protective effect in cartilage degenerative disease osteoarthritis.

Methods: *In vitro* culture of articular chondrocyte cells from rats for cytotoxicity, antioxidant, anti-inflammatory activity, cell cycle progression, transcriptional and translational expression of chondrogenic marker was studied. *In-vivo* studies of WFA at $10mg.kg^{-1}d^{-1}$ for osteochondrogenic activity in growing *Sprague Dawley* (SD) rats, dexamethasone treated growth retardation in growing rats and monosodium iodoacetate (MIA) induced osteoarthritis at Intra-articular knee joint in adult rats. Measurement of growth plate zone height and micro-computed tomography of long bones were studied.

Results: Cell viability assay showed that WFA promotes proliferation of chondrocyte cells above concentration of 1µm. Cell cycle analysis showed that WFA at 100 nM increased the S-phase of cell cycle in cultured cells and increased the metatarsal bone growth in *ex-vivo* culture. WFA improved extracellular matrix synthesis by up-regulation of Aggrecan, Collagen-II and Transcription factor SOX-9 as compared to control. It reduced the ROS generation and restored mitochondrial membrane potential at 100 nM and 10 nM concentration that was altered by inflammatory cytokines. We observed delayed chondrocyte dedifferentiation into hypertrophy by sustained expression of Collagen10, Vascular endothelial growth factor and Runt-related transcription factor-2. *In-vivo* studies showed that the supplementation of WFA increases the length of long bones; improved the trabecular architecture by increasing bone volume and trabecular number in femur and tibia as compared to control. WFA increased the growth plate height by increasing the cells in proliferating and hypertrophic zone. In chondro-protective studies we found that, WFA treatment reduced glucocorticoid (DEX) prompted growth retardation and cartilage loss of subchondral bone in MIA-treated animals.

Conclusion: We conclude from our study that WFA has the ability to increase bone length and trabecular micro-architecture of long bones in a model of catch-up growth in female rats. WFA showed therapeutic potential in osteoarthritis directed cartilage disruption and glucocorticoid induced growth arrest in SD rats.

Research funding: CSIR and ASTHI (BSC0201) Government of India.

P022

Development of assays to assess the clinical utility of tenascin-C

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Objectives

Tenascin-C (Tnc) is a pro-inflammatory extracellular matrix glycoprotein which is highly upregulated at sites of inflammation. Tnc has been implicated in the pathogenesis of a number of inflammatory disorders including as a key driver of arthritis. Serum levels of Tnc have been shown to be significantly elevated and positively correlate with disease duration in patients suffering from arthritis (Page et al., *Arthritis Res Ther,* 2012). This project aims to investigate the diagnostic and prognostic potential for Tnc in arthritis, a disease severely lacking in reliable robust biomarkers. Initially this will involve better characterising the basal expression of Tnc and its subtypes in mice.

Methods

Wild type C57BL/6J male 12, 28, 70, and 200 day old mice (n=3 mice per time point) were sacrificed and a variety of tissues harvested including tibia. RNA was extracted and RT-qPCR used to quantify *Tnc* mRNA (all splice variants) expression which was normalised to *18s* rRNA levels.

Results

Tnc mRNA expression is generally low in most tissue types sampled including tibia where it appeared to be higher at the earlier time points and then decreased with age. Brain was the only tissue to show any significant levels of *Tnc* which was maintained at all time points sampled. A large transient elevation in *Tnc* expression was also observed in the testes at the 28 day time point and which was lost at later time points.

Conclusions

Tnc expression as expected appears to be largely restricted in post-natal mice, which is consistent with the current literature. Future work will aim to continue to characterise Tnc and its subtypes in wild type mice as well as in arthritis and other inflammatory disease models. This will include using immunohistochemistry (IHC) to investigate Tnc expression at the protein level in the same panel of tissues and time points. This will perhaps prove more informative as Tnc has been shown to be expressed in some micro tissue structures such as stem cell niches which IHC will allow us to identify.

P023

Inhibition of CTX-II release by cathepsin K inhibition in vivo but not in vitro suggests that anti-resorptive therapy protects cartilage.

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Animal models and observations in humans suggest that inhibitors of bone resorption protect cartilage in osteoarthritis (OA). Part of the evidence for this is that anti-resorptives suppress release of CTX-II, a peptide fragment generated from cleavage of type II collagen. The presumed explanation has been that stabilisation of subchondral bone protects the overlying cartilage against degradation by chondrocytes. However, an alternative explanation has recently been proposed: that osteoclasts themselves release CTX-II. To distinguish between these two models, we compared the ability of a cathepsin K inhibitor to suppress CTX-II release in vivo with its ability to inhibit CTX-II release by osteoclasts in vitro.

Human monocyte-derived osteoclasts were incubated on slices of subchondral bone and overlying calcified and hyaline cartilage ('joint slices') from patients with OA. We found substantial release of both CTX-I (a bone resorption marker) and CTX-II from the joint slices. As expected, the cathepsin K inhibitor MV061194 strongly suppressed CTX-I release. However, CTX-II release was unchanged. CTX-II release was though inhibited by the MMP inhibitor GM6001. In contrast, in female beagle dogs subjected to partial medial meniscectomy, an experimental model of OA, the selective cathepsin K inhibitor MIV-711 reduced both CTX-I and CTX-II levels (to 14±1% and 20±2% respectively, compared to baseline).

Suppression of CTX-II release by cathepsin K inhibition in vivo but not in vitro suggests that the fall in CTX-II in vivo is not explained by direct suppression of osteoclastic release. Rather, our results suggest that it occurs through an indirect effect, such as protection of cartilage from the destabilising effect of increased bone turnover in the subchondral plate. Thus, the loss of subchondral bone which is an early event in OA may destabilise cartilage and induce its degradation by chondrocytes; and this process may be prevented by anti-resorptives. Furthermore, our results suggest that suppression of CTX-II by anti-resorptives is a marker of chondroprotection in vivo.

P024

What is in the Osteoarthritic bone marrow lesion?

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Introduction

Bone marrow lesions (BML) are identified by MRI imaging in many osteoarthritis (OA) patients. Comparatively few attempts have been made to correlate clinical imaging with histopathology, with changes involved in these regions needing further clarification. Preparation for standard histology using decalcification, wax embedding and sectioning loses the mineral content and 3D context. We hypothesised that approaches using scanning electron microscopy (SEM) of large tissue blocks embedded in PMMA might give more intact tissue, new insights and better correlation with clinical imaging.

Materials and Methods

Distal femur/proximal tibia samples were obtained at knee replacement from three OA cases after MRI imaging of BML with full informed consent. Tissue pieces were dehydrated in ethanol and embedded in PMMA. Block surfaces were trimmed and polished to 4000 grit silicon carbide paper. To study mineral content only, they were examined without coating by 20kV BSE SEM imaging at 50Pa chamber pressure. To study soft tissue histology blocks were stained using iodine vapour before SEM.

Results

BSE SEM of iodine stained blocks provided good imaging of all soft tissue phases. Problems were encountered near surfaces cut during surgery due to bone fragment impaction into marrow spaces, but was less of a problem near and in the BML sites, which was solved by re-cutting the block surfaces normally to generate section planes further from this initial damage, with the further advantage that both cut surfaces could be imaged simultaneously. Most normal bone marrow was adipocytic with adipocytes the major bone lining cells, frequently making and moulding trabecular excrescences. Bone volume fraction was starkly reduced in BML areas, with marrow replaced by: 1) nothing recognisable morphologically 2) dense fibrous connective tissue or 3) hyaline cartilage or fibrocartilage.

Areas of aggressive resorption were found at the periphery of BML patches and areas of calcified cartilage so deep within the bone that they could not be explained by impaction from the joint surfaces, but must have arisen by mineralisation of cartilage formed deep within the bone organ.

Conclusion

We provide 2.5D or 3D SEM histology correlated with 3D clinical imaging to demonstrate what is in the BML lesion.

P025

The Rare and Undiagnosed Diseases Study (RUDY) Platform – a novel approach to patient-driven research in rare musculoskeletal diseases.

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Objective: Research into rare diseases is increasing with growing recognition of the significant diagnostic and therapeutic care gaps. The aim of the RUDY study is to develop a web-based registry and patient-driven research platform to address many of the issues of rare musculoskeletal disease research.

Method: With involvement of key patient groups and clinicians in the early design phase, an internet-based platform, www.rudystudy.org, was created. Features include online registration; telephone consent and online capturing of patient reported outcome measures and events within a dynamic consent framework.

Results: To date 384 participants have been recruited, 335 adults and 49 children, and questionnaire completion rates are in excess of 50%. There have been no withdrawals and 2/346 have amended their consent options.

Conclusion: The RUDY platform informs the clinical phenotype with patient entry of phenotyping data within a dynamic consent framework. It is working particularly well for patients with rare diseases who may live in geographically diverse areas because it allows frequent interaction without a significant burden of travelling time and costs. Compared with more traditional study models that require face to face interactions between participants and researchers, this model enable greater numbers to be recruited and could be extended into an international database with shared data collection linked with national / regional consent processes/ approvals. Use of similar web based portals with dynamic consent may be applicable to research in other rare and more common diseases.

Funding: This study is jointly funded by the NIHR Rare Diseases Traslational Research Collaboration and the Oxford NIHR Musculoskeletal Biomedical Research Unit, University of Oxford

P026

Differences in bone density and geometry in adolescent male athletes engaged in UK popular sports: The PRO-BONE study.

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Objectives
Exercise is an effective approach for developing bone mass and adolescence is a key period to optimize bone health. However, sports specific training may have different effects on bone outcomes. This study examined the differences in bone outcomes between osteogenic (football) and non-osteogenic (swimming and cycling) sports and a control group in adolescent males.
Methods
One hundred and twenty one males (13.1±0.1 years) were measured: 41 swimmers, 37 footballers, 29 cyclists and 14 controls. Participants in the sports groups engaged ≥3 h/week in their specific sport and controls did not engage in any of these sports ≥3 h/week. Bone mineral density (BMD) and bone mineral content (BMC) were determined using dual energy X-ray absorptiometry (DXA). Hip structural analysis (HSA) applied at the femoral neck evaluated bone geometry parameters. Results were adjusted for age, height, region-specific lean mass, calcium intake and moderate to vigorous physical activity.
Results
Footballers had increased BMD at total body less head (7-9%), total hip (12-21%) and legs (7-11%) compared to the other groups and increased BMD at the femoral neck than controls (14%). Cyclists had increased BMD at the trochanter site (10%) compared to controls. Geometrical HSA showed that footballers had increased cross-sectional area (8-19%) compared to all the groups and cross-sectional moment of inertia (17%) compared to controls. Hip strength index was higher in footballers compared to swimmers (21%) and controls (38%), and in cyclists compared to controls (29%). All results above were significant at $p<0.05$. No significant differences were observed between swimmers and controls or between swimmers and cyclists at any bone outcomes.
Conclusion
The findings show that adolescent male footballers have enhanced bone density at the most skeletal sites and geometry compared to all groups. Swimmers and cyclists had similar bone outcomes and there was a trend (non-significant) for greater bone outcomes in cyclists and swimmers compared to controls.

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P027

Effect of denosumab treatment in osteoporosis patients who had long term bisphosphonate therapy

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Objectives

Osteoporosis patients who had long term bisphosphonates (BP) were commenced on Denosumab (DN) treatment. The objective of this retrospective study was to assess the effect of DN treatment in osteoporosis patients who had long term BP therapy on bone mineral density (BMD) and recurrent bone fragility fractures.

Methods

44 osteoporosis patients with history of bone fragility fractures were commenced on DN therapy after they received BP for 3-10 years. Bone mineral density was measured by DXA scan before and after completing the DN treatment. Study patients were divided into two groups based on BMD response to DN. Patients who responded to DN showed increase in BMD and patients who did not show response to DN showed either no change or decreased BMD.

Results

Osteoporosis patients who responded to DN (n=28)

64% had BP for 7-10 years and 36% for 3-6 years before they were commenced on DN. 46% non-vertebral , 42% vertebral and 10% had hip fractures before DN treatment. 64% had 25-hydroxy vitamin D levels above 50nmol/L and 57% had bone resorption marker CTx(C-terminal telopeptide) ≥ 0.2microg/L before commencing DN. 60% patients completed 3 years of DN treatment. 10% patients fractured during DN treatment.

Osteoporosis patients who did not respond to DN (n=16)

56% had BP for 3-6 years before they were commenced on DN. 62% had non-vertebral fracture before DN. 68% had 25-hydroxy vitamin D levels below 50nmol/L and 62% had CTX≥ 0.2microg/L before commencing DN. 43% completed 3 years of DN and 25% 2 to 3 years of DN. 25% patients fractured during DN treatment.

Analysis of recurrent fractures between two groups

There was no significant difference in recurrent bone fragility fracture between the two groups ($p=0.70$, chi square test)

Conclusions

36% osteoporosis patients who had long term BP showed poor response to DN by having either no change or decreased BMD.

P028

Effectiveness of measuring bone alkaline phosphatase in monitoring patients with chronic kidney disease with bone mineral disorders

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Introduction

In September 2013 we implemented the 2009 KDIGO (Kidney Disease: Improving Global Outcomes) guideline for managing osteoporosis in patients with chronic kidney disease mineral and bone disorders (CKD-MBD) in our bone metabolic clinic. According to this guideline bone alkaline phosphatase (bALP) is a reliable marker in managing osteoporosis in patients with CKD-MBD. The aim of this retrospective audit was to assess the effectiveness of measuring bALP in managing osteoporosis in patients with CKD stage 4/5/dialysis.

Methods

This was a retrospective audit of 46 osteoporosis patients with CKD 4/5/dialysis who had either a previous history of bone fragility fracture or were taking oral prednisolone. Patients had baseline calcium profile, CTx(C terminal telopeptide), P1NP(type 1 procollagen N terminal peptide), bALP, PTH and 25OH vitamin D measured and at follow up visit. Vitamin D status was corrected with the aim to achieve levels ≥75nmol/L. Those patients with 25OH vitamin D ≥75nmol/L but with high bALP(above the reference range) were given a single dose of denosumab (DN) 60mg subcutaneously. All patients were then monitored in clinic over a period of two years to assess for further bone fragility fractures.

Results

Of our patient population (n=46) the majority were female (n=36) with 72% being CKD stage 4. 72% of the patients had a history of previous bone fragility fracture. 48% of these patients had vertebral fracture, 36% non-vertebral and 30% hip fracture. 35% patients were on prednisolone and of these 75% had a history of fracture. 88% of patients had high baseline bALP and of these 73% had adequate vitamin D stores. The majority of patients (45%) had 1 injection of DN. 67% of patients had >30% reduction in bALP following their first injection. 6.5% of patients had a recurrent bone fragility fracture during the follow up period($p < 0.0001$, paired t test).

Conclusions

Study shows that bALP can be used effectively to monitor response to treatment and to select appropriate osteoporosis patients for DN treatment in patients with CKD MBD.

P029

An evaluation of pre and post-operative biochemical and haematological changes in patients with hip fracture

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Objectives

Hip fractures are common and occur in association with multiple comorbidities. Guidelines, which aim to standardise clinical care, inform management of peri-operative biochemical and haematological derangements. In this single-centre evaluation of clinical practice, we aimed to determine the frequency of pre and post-operative anaemia, acute kidney injury (AKI) and abnormalities of calcium homeostasis.

Methods

We linked routine biochemistry and haematology results to National Hip Fracture Database audit data for all hip fracture patients admitted over one year in one NHS centre. Data were cleaned and analysed in Stata 12.

Results

Linked data were available in 475 of 494 (96%) patients with hip fracture. Mean (SD) age was 83(8.8) years; 75.4% were female. 194 (40.8%) were anaemic on admission (male haemoglobin <130g/dL; female <115g/dL). 264 (55.7%) had a post-operative haemoglobin drop of >2g/dL. Mean (SD haemoglobin drop varied by operation type: intramedullary nailing 3.5(1.1)g/dL, total hip replacement 3.0(1.5)g/dL, hemiarthroplasty 2.8(1.4)g/dL, dynamic hip screw 2.7(1.4)g/dL.

On admission, 17(3.6%) had chronic kidney disease stage 4/5. Post-operatively 46(11.4%) dropped their estimated glomerular filtration rate (eGFR) by >25%. After 3 weeks, only 13(6.8%) had a persistent deterioration in renal function (amongst 191 (40.2%) with sufficient follow-up data).

Vitamin D deficiency (<30nmol/L) was seen in 240 (50.5%) and insufficiency (30-50nmol/L) in 68 (14.3%). On admission, 169 (35.6%) were hypocalcaemic (<2.20mmol/L) and 43 (9.1%) hypophosphataemic (<0.8mmol/L); the majority of whom had low vitamin D levels. A further 7 (1.5%) were hypercalcaemic and 5 (1.1%) had a low alkaline phosphatase (ALP) in the context of fracture.

Conclusion

Anaemia on admission was common. The high frequency of substantial peri-operative haemoglobin drop, together with the potential adverse effects and cost of blood products, has prompted changes in more routine use of intravenous iron. The majority of patients had stable or improved CKD stage post-operatively, suggesting current guidelines minimise AKI. Given the frequency of low ALP, guidelines are required regarding investigation of hypophosphatasia in the context of hip fracture.

P030

Icaritin anabolic function on human osteoblast via up-regulation of CXCR4.

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Objective:

To elucidate the mechanism in which Icaritin increases the anabolic function of osteoblast.

Methods:

Anabolic parameters of human osteoblast including proliferation, differentiation, migration, and mineralization were performed. qPCR and western blot analysis were performed to elucidate changes in signaling pathway. Gene knockdown of suspected signaling pathway performed and downstream pathway were analyzed. Subsequently, anabolic parameters were measured to verify if there were any changes, and if so, were they significant.

Results:

Icaritin was able to significantly increase the rate of proliferation, differentiation, migration and mineralization in human osteoblast. Q-PCR and western blot analysis showed an increase in the expression of several well established markers of bone anabolism, as well as the G-protein couple receptor (GPCR) CXCR4.

Subsequent knock-down of CXCR4 resulted in the down-regulation of BMP2 as well as Runx2. Consequently, the knock-down of CXCR4 also resulted in a decrease in anabolic phenotype previously observed. In addition Icaritin was unable to rescue the decrease in those parameters.

Conclusion:

Icaritin is a compound that is able to increase the anabolic function in human osteoblast. It does so by increase the expression of CXCR4 which lead to the downstream activation of other important downstream transcription factors including BMP2 and Runx2. In addition, the subsequent knock-down of CXCR4 resulted in a decrease in anabolic parameters observed and Icaritin treatment was unable to restore them back. This suggest that CXCR4 could be a via target for anabolic treatment of osteoporosis.

P031

Effects of sexual hormone deficiency and reposition associated to malnourishment (Cafeteria diet or protein restriction) on bone metabolism in adult female Wistar rats

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Osteoporosis is a pathology characterized by loss of bone mass leading to bone fragility and increased risk of fractures. It affects mainly post-menopausal women due to the decrease in sexual hormones; the hormone reposition therapy (HRT) treats and prevents the menopause symptoms, including osteoporosis. The diet also has a central role in bone metabolism. An adequate ingestion of protein is determinant to the maintenance of bone and muscular mass. Besides, the contemporary nutritional transition increased the consumption of high caloric and highly processed food and beverages, leading to obesity and other metabolic alterations. The objective of this study was to evaluate the effects of a low-protein diet and cafeteria diet on bone metabolism in a condition of sexual hormone deficiency with or without HRT. This study analysed control, ovariectomized, and 17β-estradiol-treated ovariectomized adult female Wistar rats fed with control, low-protein or cafeteria diet. Weight gain, body composition and bone fragility were assessed, as well as hormone assays. The results demonstrate that the hormonal deficiency (ovariectomy) affected the growth process, increasing the weight gain, body fat percentage and diminishing the bone and muscular mass, bone density and bone strength. The HRT was efficient to restore these observed parameters to the control levels. The protein deficiency of this study was sufficient to cause alterations in growth and muscular mass, diminishing those parameters. The low-protein diet negatively affected the ovariectomized animals, and the HRT was less efficient compared to the control. The cafeteria diet led to alterations in growth, increasing the weight gain, the body fat percentage and diminishing the bone and muscular mass, bone density and bone strength. Besides, the cafeteria ovariectomized group was more affected compared to the control and low-protein groups, and the HRT associated with this diet had a significantly reduced effect. In conclusion, malnourishment negatively affects bone metabolism in a sexual hormone deficiency condition, and also jeopardized the hormonal treatment. Thus, HRT should be associated with a healthy nutritional pattern.

P032

Laser Microtomy for regular Histology and advanced Histochemistry in Bone Research

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With routine methods like rotary or sledge microtomes, it is nearly impossible to cut large undecalcified hard tissue samples. Ground section technologies generate sections of less quality (especially thickness is a limiting factor) and with low throughput associated with high material loss.

Laser Microtomy is a new method to cut histologic sections of plastic embedded hard tissue. This method allows generating thin sections of large compact undecalcified bone samples by using a pulsed femtosecond laser. For the first time serial sectioning of large undecalcified bone at thickness of 10 µm is possible. We will show sections of selected bones (sheep spine, rat and mouse femur and tibia, cow tail) and further ones including implants, such as polymers.

One important aspect of establishing the new method is to integrate it into the routine processes of an existing lab. Therefore, we tested common stains like Hämatoxilin and Eosin (H&E), Masson Goldner Trichrome (MG), Levai Laczko, or Sandersons Rapid Bone Stain with van Gieson (SRS + VG). The stains show the expected results for each stain, comparable to stainings performed on sections prepared with a classical microtome. The results show clear structured and stained samples. 10µm sections of undecalcified bone allow analyzing structures in more detail.

Also special detection of enzyme activity inside bone samples like Tartrate-Resistant Acid Phosphatase (TRAP) for osteoclast activity was tested. Temperature sensitive structures (enzymes in osteoclasts) are still intact and can be demonstrated by TRAP staining. This shows that the laser cutting does not impair the molecular structure of the cells and the tissue. Laser microtomy opens a new range of possibilities in analyzing hard tissues. Stainings the pathologist is used to work with in routine, can be transferred to laser sections, sometimes the protocol has to be slightly adapted.

P033

Impact of fracture risk assessment tools on proportions of women without prior fracture identified at risk and treated - lessons from the SIGN guidance

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Recent guidance from the Scottish Intercollegiate Guideline Network (SIGN) proposes that women aged 50 years and older with a ≥10% risk of fracture, in the absence of a prior vertebral or hip fracture, should have treatment if a subsequent BMD scan shows T-score osteoporosis. We have compared the impact of the use of FRAX or QFracture on the proportion of women, without a prior fracture, that would be identified as needing a BMD scan and subsequent therapy.

The number of women in Scotland with a fracture risk of 10% or more were estimated using the distributions of QFracture incidence and FRAX probabilities in an analysis of the THIN database[1] adjusted for the age demography of women in Scotland.

In a total population of nearly 1 million women, 275,600 (28%) women without prior fracture would be identified as having a 10-year FRAX probability of 10% or more; the proportion increased from 2% at ages 50-59 years to 58% at ages 80-89 years. In contrast, only 2.7% of women would be identified by QFracture (ranging from 0% at younger ages to 16% at 90-99 years). The number of women subsequently eligible for treatment would be 81,700 (8.2%) with FRAX, rising from 0.2% of the population at 50-59 years to 32% at 90-99 years; the proportions were much smaller with QFracture with only 12,300 (1.2%) identified for treatment, ranging from 0% of the population at 50-59 years to 10% at 90-99 years.

We conclude that the use of the QFracture algorithm results in a marked reduction in the treated population, reflecting well-demonstrated deficiencies in the calibration of QFracture for fractures other than hip fracture. The use of QFracture will result in little or no impact on the overall incidence of fractures in women without a prior fracture compared to the use of FRAX.

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P034

The effects of parathyroid hormone peptides on the peripheral skeleton of postmenopausal women: A systematic review

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Novel anabolic therapies for bone are in late-stage clinical development and their effects on fracture risk in the peripheral skeleton will be of particular interest. The effects of parathyroid hormone peptides, the only currently available anabolic agents, on the peripheral skeleton have received relatively little study. We therefore wished to document the effects of these peptides on the peripheral skeletal measures by DXA and/or 3D imaging techniques in postmenopausal osteoporosis.

We undertook a systematic review of English articles using MEDLINE, Scopus and the Cochrane Controlled Trials Register (all accessed 21st July 2014). Additional studies were identified through searches of bibliographies. Studies included those comparing PTH peptides (1-34 and 1-84 PTH) with placebo, with anti-osteoporotic treatments and in combination therapies. Participants had to be postmenopausal women and outcomes included areal or volumetric bone mineral density (BMD) and measurements of bone microarchitecture at peripheral sites, such as the radius and tibia.

The heterogeneity between studies, regarding the treatment dose, duration measured sites, prevented grouped meta-analysis. Both PTH peptides were associated with trends to decrease aBMD within radial sites but, while these effects were often numerically greater, they were usually not significantly different from changes observed during placebo exposure. At cortical sites within the radius, both PTH peptides showed significant decreases in aBMD compared to treatment with the anti-resorptive agents, alendronate or denosumab. At the radius and tibia, cortical vBMD was lower in patients receiving 1-34 PTH compared to denosumab; cortical porosity increased in the tibia and radius during 1-34 PTH, but remained unchanged during denosumab.

PTH peptides combined with a variety of anti-resorptives appeared to attenuate rates of loss seen with PTH peptides alone, but showed non-significant trends for larger decreases in aBMD at the radial shaft compared to the anti-resorptive alone. Combination with denosumab confers small increments in cortical measures and bone strength, but the clinical significance is unclear.

This systematic review acts as a reference point for the comparison of new anabolic therapies to existing PTH peptide agents at peripheral skeletal sites.

P035

The adiponectin receptor agonist AdipoRon, activates osteoblast differentiation markers and promotes autophagy in vitro

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Adiponectin receptors (AdipoR) mediate the positive effects of the beneficial adipokine adiponectin, such as promoting resistance to neurodegeneration, diabetes and prolonging lifespan. Consequently, novel agonists to both AdipoR1 and AdipoR2 are sought as novel therapeutics to maintain good health in later life. While genetic manipulation of adiponectin has suggested a complex regulatory system exists controlling adiponectin signalling within the skeleton, the effects of targeting AdipoR specifically have not been reported. AdipoRon is the first selective, orally active, synthetically derived small-molecule agonist of both AdipoR1 and AdipoR2 to be developed. Treatment of the diabetic *db/db* mouse line with AdipoRon ameliorates insulin resistance and glucose intolerance, improves exercise endurance and extends lifespan. We hypothesize that treatment of osteoblasts with AdipoRon will improve overall formation and function, and may form a potential bone anabolic to improve bone mass in vivo.

The osteoblast 2T3 cell line was treated with increasing concentrations of AdipoRon (20-50uM) for 24 and 48 hours. PCR analysis showed that Runx2, an early marker for osteoblast differentiation, increased with increasing AdipoRon treatments (x8 fold $p<0.05$) suggesting that AdipoRon stimulates initial osteoblast differentiation.

In tumour cells, activation of the adiponectin receptors has been show to trigger autophagy and increased cell survival mechanism. Autophagy is an ageing-related mechanism known to be dysregulated in the elderly, and underlying a number of age-related pathologies such as neurodegeneration and arthritis. Activation of autophagy requires the formation of complex vesicular intracellular structures, where LC3 protein expression and conversion to LC3 II forms a critical step. Treatment of 2T3 cells with AdipoRon as above, increased LC3 levels and conversion by Western blot (x2 fold, $p<0.05$), suggesting that adiponectin receptor stimulation also triggers autophagy in bone cells.

These results suggest that stimulation of AdipoR1 and R2 buy the novel adiponectin agonist AdipoRon, may activate osteoblast differentiation and improved cellular survival through enhanced autophagic flux.

Funded by Arthritis Research UK

P036

Ex vivo model of human cancer-bone microenvironment

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Bone is the most common site for tumour metastasis, particularly in breast and prostate cancers where ~70% of patients show evidence of metastatic bone disease. Consequently, a large field of research has evolved to specifically study cancer-bone disease, along with a large number of different animal models. These include inoculation of human or mouse tumour cells at extra-osseous sites e.g. heart, or implanted directly into skeletal sites. The limitations of *in vivo* bone-tumour models include a common mixed approach using human cells in mice, using immuno-compromised mice which do not clearly represent the contribution of the immune system within the bone-tumour niche, and the wide-ranging inherent differences between mice and humans.

To more accurately re-create the human bone-human tumour microenvironment, and importantly, replace the use of animals in unnecessary or poorly validated model systems, we aimed to develop an *ex vivo* assay mimicking the tumour-bone cell interactions which occur following cancer metastases to skeletal sites.

Fresh trabecular bone samples were collected following hip replacement surgery under approved Ethical and HTA procedures. Uniform areas of trabecular bone were created approx. $5mm^2$, and cultured with PC3-EGFP prostate cancer cells +/- HS-5-mCherry human bone marrow stromal cells. Cells were inoculated to the living bone by either direct plating upon the bone surface, insertion into small cores within the bone or by introducing the cells in a matrigel suspension. Following culture for 7-10 days the tumour-bone co-culture was processed for histomorphometric analysis.

Viable bone cells were clearly visible (osteoblasts, osteoclasts) and abundant fat deposits, common in ageing human bone. Importantly, tumour cells were observed adhered to the bone surface and occupying marrow spaces within the bone network when introduced within matrigel, suggesting the capacity to grow and colonise the bone niche. However, few GFP+ tumour cells were seen when inoculated by other means. Our assay has used human intact bone and human tumour cell lines to model the bone-tumour niche seen in patients with metastatic bone disease and suggests a potential alternative to the use of animal models in cancer-bone research.

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P037

Predicting hip fracture risk from sideways falls using computer modelling techniques

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Objectives
Annually, major morbidity from falls includes hip fracture in 800,000 individuals throughout Europe[1]. This project aims to use an integrated computer modelling approach that combines finite element analysis (FEA), micro computed tomography (uCT) and multibody dynamic analysis (MDA), to create more accurate finite element (FE) models of hip fracture by facilitating the inclusion of appropriate bone parameters and boundary conditions.

Methods
Proof of concept study: a general FE model of a femur[2] was constructed and loaded with impact forces of magnitudes previously found to cause failure in the proximal femur. These acted alone or in conjunction with hip abductor muscles that were given arbitrary but plausible force values.

Five complete cadaveric proximal femora from elderly individuals were uCT scanned at 68-73 microns. Custom written Matlab scripts divided scans into bone cubes which were analysed in BoneJ to determine trabecular orientation. Further custom scripts mapped trabecular orientations in three dimensions to explore variation between individuals and femoral regions.

MDA simulations of sideways falls are being carried out in the GaitSym simulator which uses a forwards dynamics global optimization system[3]. Static matches have been performed and dynamic simulations are being run to obtain plausible hip muscle forces/activation patterns that occur during sideways falls.

Results
• The proof of concept study suggests contraction of hip abductors coincident with fall impacts might increase hip fracture risk at reduced impact loads. This shows a need to derive hip muscle forces/activation patterns from MDA to investigate their effects properly.
• Trabecular orientations have been quantified and mapped from multiple uCT scans of complete proximal femora. Data now exists for those seeking to create general models of trabecular orientation in the complete proximal femur.
• Future work will include incorporating MDA data and trabecular orientations in FE models of the scanned femora.

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P039

Relating Biological signals to Biomechanics in High Tibial Osteotomy

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Introduction

Mechanical mechanisms underlying joint degeneration are not understood. High tibial osteotomy (HTO) surgery realigns varus knee to treat joint disease. Glutamate is mechanically regulated in bone, activating glutamate receptors (GluRs) in joints to cause inflammation and degeneration, representing a mechanism through which joint loading influences pathology. To evaluate whether glutamatergic signals correlate with joint loading, biomechanics and biological signals were measured pre and post operatively in HTO patients.

Methods

Bone cores were taken (Anterior/Posterior Medial/Lateral (AM/PM/AL/PL)) from proximal tibia (HTO surgery and plate removal) and gait assessments (pre-surgery and 9-12mths post-op) performed in 5 patients (48.9±6.1yrs;88.9±24.3kg;1.75±0.17m).

Bone core RNA was reverse transcribed and GluRs (NR2D, GRIK4), glutamate transporter (EAAT3) and reference (HPRT1) genes quantified using absolute quantitative PCR. Lower limb joint biomechanics were quantified (Visual3D, C-Motion), using motion analysis (Qualisys) and force-plates (Bertec) and Cardiff Classifier used to discriminate between pre and post- HTO. The top 13 discriminating biomechanical variables were correlated with gene expression, extent of varus and pain (Pearson's Correlation, SPSS).

Results

Knee varus angle pre and post HTO significantly correlated with biomechanical (Knee Adduction Angular Impulse (KAAI) at mid-stance,$p=0.027$; Knee adduction at knee adduction moment (KAM) peak1&2, $p<0.001$) and gene expression (NR2D-AM,$p=0.042$; NR2D-PL,$p=0.024$; EAAT3-PM,$p=0.041$) data. Top-ranked variables that discriminated between pre and post-HTO included: KAAI (heel strike/mid stance), Knee adduction at KAM peak1&2, KAM peak2, hip rotation angle at KAM peak2, Knee adduction ROM and NR2D-AM expression. Biomechanical variables correlated with biological signals: KAAI (across gait cycle) with EAAT3-PM($p=0.047$) NR2D-PL($p=0.033$) and EAAT3-AL($p=0.026$); Knee adduction angle at KAM peak2 with NR2D-PL($p= 0.021$) and EAAT3-PM($p=0.021$); Ankle moment at KAM peak 1 with NR2D-PL($p=0.044$), EAAT3-AL($p<0.001$), and GRIK4-PM ($p=0.044$). EAAT3-AL expression correlated with pain($p=0.015$).

Discussion

This is the first report of longitudinal, biological and biomechanical data pre and post HTO surgery. Extent of varus correlated with biomechanical (KAAI/knee adduction angle at KAM peak1), and biological (NR2D/EAAT3) factors. Biomechanical factors that changed most pre and post HTO surgery (KAAI, Knee adduction angle) correlated with expression of GluRs (NR2D/GRIK4), and EAAT3. EAAT3 expression correlated with pain. Thus HTO surgery may cause mechanical regulation of glutamatergic signalling in sub-chondral bone to influence pathology.

P040

Clinically available scanning techniques are a potential detection tool for high density mineralised protrusions (HDMP) in human knees joints

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High density mineralised protrusions (HDMPs) from the mineralising front into hyaline articular cartilage (HAC), originally discovered in horses have been confirmed in human hip joints. Thought to be naturally occurring, these phenomena possess mineral concentrations greater than any healthy joint structure and have the potential to fragment. Thus, their presence would likely influence the biomechanical performance of surrounding tissues and they have been associated with osteoarthritis (OA).

Incidence, location and morphology of HDMPs in human knees were assessed by two methodologies: ***In-vivo***: MRI data of patients with Alkaptonuria (n=36; age=19-67) were acquired sagitally (in-plane resolution=0.58mm) and potential protrusions were assigned a confidence value (1-5; 1=least confidence), representing potential for misinterpretation. ***Ex-vivo***: Knees (n=11) from 8 cadavers (age=74-97) were imaged isotropically (resolution=0.26mm) by dual echo steady state MRI. Size and location of potential protrusions were recorded. Knees were assessed for OA using the Kellgren-Lawrence (KL) scale.

All knees had signs of OA (KL score ≥1). A total of 216 potential HDMPs were identified across both studies (*in-vivo*=180; *ex-vivo*=36), with ≥1 reported in 10 out of 11 cadaveric subjects and in all patients. Ninety-two percent of those noted *in-vivo* had a confidence score ≥3, indicating a low likelihood of misinterpretation. Distribution was variable, with potential protrusions noted in all areas of the joint. They were observed in isolation and in small clusters. The ratio of femoral to tibial protrusions *in-vivo* was 4.6:1 compared with 1.77:1, *ex-vivo*. The percentage of protrusions found in regions central to articulation was 50 *in-vivo* and 75 *ex-vivo*. The mean width and depth of protrusions within HAC were 1.67mm and 1.72mm. However, morphology varied considerably.

Regions central to articulation typically experience the greatest stress within the knee. It is likely that the many HDMPs observed here would be subjected to fragmentation, at the detriment of surrounding HAC. This may account for clustering and is suggestive of a role in progression of arthropathy. Morphological variability may be indicative of different pathophysiological forms and stages. Crucially, these data suggest radiologic detection of HDMPs is possible with clinical technology. Moreover, it should be considered as a biomarker for predicting joint destruction.

P041

Pitfalls in assessesment and management of osteoporosi in a secndary care centre

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OBJECTIEVE

To assess whether we properly assessing fragility fractures who attending our fracture clinic in a seconsary care centre for secondary prevention of osteoporosis.

METHODS
Retrospective study of 72 patients with wrist fractures for our study.the patients were selected from our fracture clinics during 3 months period from 01/10/2015-31/12/2015.we selected patients with wrist fracture above 50 years. The patient details were obtained from Clinic slips, patient notes,cyberlab.PACS and online patient notes. The permission for study was obtained from our audit department

RESULTS

We had 9 males and 63 females.tha age ranged from 50-93.the mean age was79.Out OF 72 patients 17 had operation fro fixing diatal radius fractures and 55 were treated nonoperatievely.there were 10 patients from nursing home and care home where there is high risk of falls. There were 21 patients with high risk including smoking,alcoholisum,recurrent falls.Only two patients were on bone protective agents.

Out of 72 patients only 16 patients were assessed and treated for osteoporosis.14 was referred for Dexa and two for alendronate.The referral to GP for assessment and treatment of osteoporosis is also poor.

DISCUSSION

Only 13 of 47 patients had investigation/management of osteoporosis in a secondary care setup .It is a ‘Bermuda Triangle' comprising orthopaedists ,primary care physicians and osteoporosis experts, into which the fracture patient disappears without trace. Literature shows that past history of fracture after the age of 45 years, but only 28% were receiving bone-protective drugs. We are lagging behind in osteoporosis management for other fragility fractures were we only gave treatment to 20.8% people where national average is well above it
NHFD states 79.5% of hip fractures had DEXA referral/16.5% assessed but no action taken.We were having good results for us with 99.5% compliance for hip fractures assessment for bone protection.

- Reasons for poor compliance for us

-Busy fracture clinic,
-fracture liaison service only available to some clinic,
-poor coordination between primary and secondary care
-Lack of structured and automatic referral process for risk assessment and treatment for osteoporosis

CONCLUSION

Unless we create fracture liaison service throughout our country or national osteoporosis data base our primary prevention of hip fractures will fail miserably

P042

Investigating phosphatidylserine metabolism in Lenz-Majewski syndrome

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Background: Lenz-Majewski syndrome (LMS) is a rare sclerosing bone dysplasia associated with intellectual disability and multiple congenital anomalies. It is characterized by progressive skeletal hyperostosis particularly of the cranium, vertebrae and diaphysis of the tubular bones leading to growth restriction and fusing of fingers and toes, together with skin and brain defects. The condition is caused by an anomaly in the production of phospholipids, which are an essential component of all human cell membranes. We previously identified the genetic basis as de novo gain-of-function (GOF) mutations in *PTDSS1*, which encodes phosphatidylserine synthase (PSS1).
Objectives: The GOF mutations activate the enzyme and reduce end product inhibition, however, the exact mechanism leading to hyperostosis is not yet understood. Current studies seek to clarify the key events at a cellular and biochemical level.
Methods: LMS patients and control cell lines as well as a CHO cell line with or without the LMS R95K mutation have been investigated using electrospray ionization mass spectroscopy techniques to generate their phospholipid profiles. To investigate mineralisation, LMS and control fibroblasts have been used to investigate propensity to calcification using a cresolphthalein assay. Matrix vesicles (MV) were also isolated from cell culture media by ultracentrifugation and analysed for PS content using flow cytometry.
Results: Lipid studies in CHO cells confirmed a generalised overproduction of PS species. Bone mineralization is initiated by MV containing nucleation sites consisting of PS and Annexin A5. We isolated MV from the cell culture media of human control and LMS fibroblast. Notably, LMS4 fibroblast-derived MVs were enriched with PS in comparison to control fibroblasts-derived MV. Next, we stimulated fibroblasts with elevated calcium and phosphate and observed rapid cell calcification in vitro as detected by Alizarin Red staining and cresolphthalein assay.
Conclusions: Our findings show that LMS mutations result in a disturbance to the normally tightly regulated production of PS. Furthermore, we found that fibroblasts secrete MVs which were enriched with annexin/PS complexes that could trigger early mineralization. We intend to go on to use our model systems to investigate potential new therapies for disordered bone metabolism, which may have implications for osteopetrosis to osteoporosis and osteoarthritis.
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P043

Increases in proximal femur bone strength estimated using finite element models from computed tomography scans following brief, high impact exercise in older men

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Brief, hopping exercises modestly increased proximal femur bone mass in older men, with proportionately larger localised increases in cortical mass. As localised thinning of cortical bone is associated with increased risk of osteoporotic hip fracture, these localised changes could more substantially affect bone strength. This study thus aimed to determine the influence of these hopping exercises on bone strength estimates from finite element models that take into account bone distribution, based upon computed tomography (CT) and dual X-ray absorptiometry (DXA) scans.

We analysed proximal femur DXA and CT scans of exercise and control legs, at baseline and after a year of unilateral hopping exercises, in 33 men aged 65-80y. From DXA scans, estimates of strength and stiffness in walk and fall configurations were calculated. From CT scans, estimates of strength in 2 walk (-3 and 18º anteroposterior rotation; both 0º mediolateral rotation) and 4 fall configurations (anteroposterior and mediolateral rotation of 0,0; 0,30; 30,0 and 30,30º respectively) were calculated. Repeated measures ANOVA was used to detect significant effects of time (pre vs post exercise), leg (exercise vs control) and their interaction.

Mean strength based on CT scans increased by 6-9% in the exercise leg and 1-7% in the control leg. Effects of time were significant in all the fall configurations ($0.001 < P < 0.012$) but not walk configurations ($0.083 < P < 0.091$). Responses did not differ significantly between legs ($P > 0.158$). The minimum strength was most often in the 30,30º fall configuration. This minimum strength increased over time ($p=0.002$) from 3300 (125) to 3514 (176) N in the exercise leg and from 3265 (136) to 3470 (170) N in the control leg but did not differ significantly between legs. There were no statistically significant effects on DXA based bone strength estimates.

CT based estimates of proximal femur bone strength in fall configurations increased following brief hopping exercises in older men. Increases in the control leg as well as the exercise leg could represent cross-education effects or increased loading to the control leg, either during the exercise or as a consequence of neuromuscular adaptation. Exercise could reduce the risk of hip fracture following a fall.

P045

Ubiquitin-protein ligase UBR5 suppresses heterotopic tendon/ligament ossification

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Spatiotemporal bone development is tightly regulated to grow and maintain a functioning skeleton. However, in certain diseases heterotopic ossification (HO) can occur in soft tissue. Our studies into the N-end Rule Ubiquitin-protein ligase UBR5 revealed its role in suppressing murine heterotopic tendon/ligament ossification and hypothesise that UBR5 utilises Hedgehog signalling to influence tendon homeostasis.

Micro-CT (uCT) analysis of Prx1-Cre;Ubr5fl/fl mutant (n=9), but not control Prx-Cre limbs (n=6), revealed progressive tendon/ligament HO from as early as six weeks of age. uCT signals were present within multiple tendon/ligament sites within the lower limb (patellar, Achilles and foot). Histological analysis identified in Ubr5mt, but not Prx-Cre controls, chondrocyte-like cells within the tendon midbody (n=4), von-Kossa positive signals (n=3) and toluidine blue staining (n=4). Together these observations indicated the HO to be potentially endochondrial in nature.

Our work in Drosophila and other murine tissues indicates UBR5 as an important regulator of stem/progenitor cell function and regulator of Hedgehog signalling. Intra-peritoneal injection of Cyclopamine, an antagonist of canonical and agonist of canonical Hedgehog signalling, dramatically enhanced HO in Ubr5mt animals (>50% increase, n=4) as well as increase the volume of normal sessamoid bones at the front of the foot in Prx-Cre controls (>30%, n=4).

Taken together we concluded that loss of UBR5 function resulted in type of HO that could be further enhanced by Cyclopamine-mediated perturbation of Hedgehog signalling. Based on our studies in articular cartilage, we hypothesise that increased non-canonical Hedgehog signalling caused by both loss of UBR5 function and Cyclopamine treatment is promoting chondrogenesis within the tendon. Mechanistically, this may be occurring via conversion of tenocytes into chondrocytes (metaplasia) and/or production of chondrocytes by tendon stem/progenitor cells.

These findings highlight a potential therapeutic opportunity for HO prevention/treatment, based upon the previously unknown relationship between UBR5 function, non-canonical Hedgehog signalling and tendon homeostasis.

P046

Involvement of the PKR Signalling Pathway in the Development of Post-traumatic Osteoathritis in the Mouse

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Introduction

Joint injuries such as anterior cruciate ligament (ACL) rupture can result in acute inflammation in the knee followed by post-traumatic osteoarthritis (PTOA). Protein kinase R (PKR) has been implicated in the pathogenesis of arthritis, where abnormal mechanical load and pro-inflammatory signals lead to over-activation of PKR and joint degeneration. In addition, we have shown that loss of the PKR inhibitor, $P58^{IPK}$ in mice leads to degeneration in the knee. This study investigated activation of the PKR pathway following induction of PTOA in vivo and assessed whether inhibition of the PKR pathway affects disease progression.

Materials & Methods

Right knees of anaesthetised 12-week-old C57Bl6 mice were loaded once at 12N to rupture the ACL and the effect of intra-articular injection of a PKR inhibitor (0.5µg/kg) or carrier immediately following ACL rupture (n=4) was determined. Mice received analgesia prior to loading and procedures complied with the Animals (Scientific Procedures) Act 1986. Mice moved freely after loading. Mice were culled at 3-weeks and knees x-rayed, processed for histology and scored (OARSI) for degeneration. Phosphorylated PKR was localised by immunohistochemistry.

Results

Joint degeneration (cartilage loss, subchondral bone plate thickening, and large osteophyte formation) and significant inflammatory changes in the joint capsule were observed in loaded knees. ACL rupture caused a 1.8-fold increase ($p<0.01$) in total OARSI score compared with the contralateral knee. In loaded knees, where extensive inflammatory infiltrate was present, strong staining for phosphorylated PKR was evident. Inhibition of PKR at the point of ACL rupture appeared to worsen the degeneration with a 3-fold increase in OARSI score compared with the contralateral knee ($p<0.01$).

Discussion

An acute inflammatory response, concomitant with cartilage and bone damage occurred rapidly following ACL rupture providing a model of human PTOA. Inhibition of PKR at the point of injury resulted in an increase in degeneration. This study is the first to reveal a critical role for PKR in the immediate inflammatory events following ACL rupture, potentially implicating the pathway in the pathogenesis of PTOA. Defining the window of opportunity for therapeutic interventions will assist in testing potential treatments that limit chronic joint degeneration.

P047

Free vitamin D concentrations reflect racial and geographic differences in total 25OHD

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Background: Total circulating 25-hydroxyvitamin D (25OHD) is used as a marker of vitamin D status. It is unclear whether free 25OHD is a better marker than total 25OHD, especially in light of reported genetic and racial differences in concentrations of vitamin D binding protein (DBP).

Methods: In geographically and racially diverse samples of adult males (n=1057) from the United States, United Kingdom, and The Gambia, we compared circulating total 25OHD to directly measured free 25OHD and calculated free 25OHD. DBP peptide concentrations were assessed with proteomic methods, and DBP concentration was measured by multiple monoclonal and polyclonal immunoassays.

Results: Total 25OHD correlated strongly with directly measured free 25OHD ($r = 0.84$). Genotype determined the molecular forms of circulating DBP, and different immunoassays yielded divergent DBP concentrations, affecting calculated free 25OHD and apparent free 25OHD racial differences. When measured by a monoclonal assay, mean DBP in subjects of African origin was approximately 50% lower than in whites, whereas DBP concentration was nearly identical in all groups when measured by polyclonal DBP antibodies or proteomic methods. Free 25OHD concentrations, directly measured or calculated from polyclonal DBP assays, were lower in African Americans than in US whites, whereas Gambians had higher total and free 25OHD than UK whites (both $p<0.001$).

Conclusions: DBP concentration, and hence calculated free 25OHD, differs by DBP assay. Previously reported racial differences in DBP concentration are likely due to monoclonal assay bias, as there was no racial difference in DBP concentration by any other method of measurement. Free 25OHD, as directly measured or calculated based on polyclonal DBP assays, was lower in African Americans than US whites, reflecting total 25OHD. This confirms that many African Americans have low 25OHD levels and the utility of total 25OHD measurement in assessing vitamin D status in the general population, irrespective of race or DBP genotype.

P048

A study of the role of ghrelin and ionic mechanisms in adipogenic differentiation of bone

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Ghrelin is a gut peptide involved in growth hormone secretion, and has recently been shown have local (auto/paracrine) effects on diverse processes such as proliferation and differentiation, including in bone1. Others have shown that cell membrane potential controls differentiation of osteoblasts and adipocytes, through unclear ionic mechanisms2. Therefore, we aimed to investigate the possible involvement of ghrelin in autocrine control of cellular trans-differentiation of an osteoblastic cell line into adipocytes, and whether ionic mechanisms play a role in regulating pathological adipogenesis.

As a model we used 7F2 cells, an osteoblastic cell line from p53-/- mice, which can be differentiated into adipocytes with addition of dexamethasone, indomethacin and ascorbic acid to the normal medium. After 7 days of treatment, mRNA expression was assessed for markers of osteoblastic (osteocalcin) and adipogenic (PPARγ) differentiation. Lipid content of cells was quantified using oil red O stain, and dye extraction/absorbance measurement.

We identified that two ion channels, Kir6.1, and KCa1.1 were expressed at the mRNA level both in 7F2 cells and in cells differentiated into adipocytes, although the KCa1.1 signal was higher in untreated 7F2 cells. Components of ghrelin signalling, i.e. Ghrl, Mboat4 and several isoforms of Ghsr, were also expressed at the mRNA level both in 7F2 cells and cells induced to become adipocytes. Ghrelin treatment of 7F2 cells appeared not to affect cell number, but further promoted the lipid content of 7F2 cells treated with adipogenic medium.

These preliminary results support our hypothesis that ghrelin influences trans-differentiation into adipocytes. Our next experiments will consist of further investigating ghrelin's effects on adipogenesis, and trying to understand the role of ion channels in this process. These results will provide a better understanding of the mechanisms underlying pathological adipogenesis, which is an important step for the search for therapeutic strategies.

1. Leite-Moreira AF & Soares J-B (2007) Drug Discov. Today, 12:276-288
2. Sundelacruz S et al. (2008) PloS One, 3:e3737

P049

PDGF: a more potent osteoinductive factor than BMP2 in a bioengineered model of pathological ossification

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Heterotopic ossification (HO) is a debilitating condition defined by the rapid formation of bone in soft tissues. What makes HO fascinating is firstly the rate at which bone is deposited, and secondly the fact that that the bone formed is structurally and compositionally similar to healthy adult bone. Even though the exact triggers leading to HO remain largely unknown, there is growing evidence implicating the post-trauma inflammatory response as an initiator. To further understand the role of inflammation on this debilitating condition we utilised an established three-dimensional skeletal muscle model to act as a novel *in vitro* test bed. We identified that C2C12 myoblasts cultured in 3D divided into two distinct populations, myogenic cells and undifferentiated "reserve" cells. Gene expression analysis of myogenic and osteo-regulatory markers confirmed that "reserve" cells were primed for osteogenesis, but had a reduced capacity for myogenic differentiation. Osteogenic differentiation was significantly ($P<0.05$) enhanced in the presence of platelet-derived growth factor (PDGF), bone morphogenetic protein-2 (BMP2) and transforming growth factor-β (TGFβ). Alizarin red staining identified that PDGF promoted significantly ($P<0.05$) more mineral deposition than the current gold standard, BMP2. Using flow cytometry analysis we were able to correlate osteogenic differentiation with conversion to a newly identified Sca-1^+/$CD73^+$ phenotype. Finally, we show that PDGF-induced osteogenic differentiation could be blocked in the presence of the pro-inflammatory cytokines, tumour necrosis factor-α (TNFα) and interleukin-1 (IL1). In conclusion, the present study identified that PDGF released at the post-trauma environment is likely to represent an early factor triggering pathological ossification in skeletal muscle. Pro-inflammatory cytokines localised to the site of trauma are able to mediate PDGF-induced differentiation, consequently providing a potential way of limiting the formation of pathological bone in skeletal muscle. This has considerable implications when one considers the current use of non-steroidal anti-inflammatory drugs (NSAIDs) in HO prophylaxis. These results may also have additional importance for the induction and control of bone formation for the regeneration of hard tissues.

P050

Identification and characterisation of extracellular vesicles and uptake events in resorbing osteoclasts using TEM tomography

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Aims: During bone or dentine resorption osteoclasts form distinct membrane domains: the basolateral domain, the ruffled border (RB), and the functional secretory domain (FSD). At the RB the resorption pit is acidified, and osteolytic enzymes are exocytosed through fusion of vesicles with the membrane. Degraded bone is taken up in vesicles and transported to the FSD where it is secreted by an unknown process. There have been few studies attempting to characterise vesicles in proximity of these membrane domains. Given their small size, light microscopy alone is insufficient to visualise these vesicles. Using transmission electron microscopic (TEM) tomography, the aims of this study were to characterise (1) exocytosis at the FSD and RB and (2) uptake events at the RB.

Methods: Dentine discs with actively resorbing rabbit osteoclasts were fixed and processed for TEM using standard techniques. 200 nm thick sections were imaged in a JEOL JEM-1400Plus TEM. Tilt series (+/-60 degrees) around the structure of interest were acquired by capturing a 2-dimensional image at each degree. 3-dimensional (3D) tomograms were constructed from this image set and regions of interest rendered using Amira software. The resulting rendered models gave insight into the 3D relationships of the RB and FSD membranes with adjacent vesicles.

Results: (1) We observed intra- and extracellular vesicles of similar morphology (ranging from 100-135 nm) without visible content in contact with the FSD. Close to the RB, we observed vesicles of varying dimensions (ranging from 45-200 nm) without visible content. Some of these vesicles retained contact with the RB, suggesting recent fusion events. (2) Structures similar to clathrin-coated uptake pits were visualised at the RB; with sizes ranging from 150-200 nm and membrane thickness of 20-25 nm.

Conclusions: Using TEM tomography, we visualised and characterised previously unreported extracellular vesicles at the RB and FSD, and found evidence supporting clathrin-mediated uptake of degraded material at the RB. We will further characterise such vesicles by combining TEM tomography with high resolution immunolocalisation experiments. Such studies will help to provide insight into osteoclast trafficking mechanisms.

P051

Circulating microRNA in metabolic bone diseases-Osteoporosis

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Osteoporosis is the most common age-related bone disease. It is clinically symptomless until the first fracture happens. Circulating microRNAs have been used successfully as novel biomarkers to assess health status and progression of complex diseases. A recent review highlighted the involvement of microRNAs in the control of bone formation and remodeling. Most of these studies have been done on animal models, but few on human blood samples.

This research aims to identify circulatory microRNAs associated with osteoporosis in a test group of patients using advanced PCR arrays. The identified potential biomarker microRNAs will be validated using individual clinical specimens.

Ethical approvals was obtained prior to patient recruitment. Patient blood samples were assigned to four groups: osteopenia (T-Score <-1 and >-2.5 SD), osteopenia with fracture, osteoporosis (T-Score ≤ -2.5 SD) and osteoporosis with fracture. RNA was extracted using QIAGEN miRNeasy kits and miRNA expression profiling was performed using the Qiagen Human Serum & Plasma 384HC miRNA PCR Array kit, with data analysis carried out using Qiagen software.

We investigated the expression of microRNAs in sample pools from osteopenia and osteoporosis patient groups. A panel of 49 differentially expressed miRNAs (up or down by >3 fold) between osteopenia and osteoporosis patient groups was identified. So far miRNA expression on selected number of individual clinical samples from these groups were performed using 26 differentially expressed miRNAs (by >4 fold) by qRT-PCR. Seven miRNAs: miR-215-5p, miR-1193, miR-99a-5p, miR-100, miR-373-5p, miR-4516 and miR-122-5P, showed a significant difference by > 2 fold and p value range from <0.005 to <0.05 between the two groups. The p value was calculated using Mann Whitney test (nonparametric statistic).

Our results show that the small RNA can be successfully isolated and identified from osteoporosis patients' blood samples and that a number of miRNAs are differentially expressed. Future work is aimed at validating identified up or down differentially expressed miRNAs in a different cohort of clinical samples; to understand the role of the identified differentially expressed miRNAs in pathogenesis and to assess whether they can be used as biomarkers for osteoporosis.

P052

Hormone and Hormone Responsive Stress-Related Molecules Involved In Osteoporosis in Post-Menopausal Women

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Osteoporosis is the commonest bone disease worldwide, and is symptomless until the first fracture occurs. Osteoporosis shows increased incidence in post-menopausal women where estrogen deficiency plays a significant role in its development (Gass, 2006).
Using a combination of subtractive cDNA libraries and microarray analysis, a panel of estrogen dependent mRNAs/proteins was identified, including transcription factor, XBP1/1S, protein disulphide isomerase, AGR2 and heat shock protein HSP90/GP96. Not only are these proteins under estrogen regulation (AGR2, XBP1) or as an estrogen receptor chaperone (HSP90/GP96), but they are also associated with the ER-stress-UPR signalling pathway. The aim of this project is to understand the interplay between reduced levels of estrogen, reduced ER stress response and the development of osteoporosis by investigating the effect of steroid hormones on mRNAs / proteins of the ER stress-UPR pathways and on their microRNAs regulators in osteosarcoma (osteoblast-like) cell lines. Identified proteins/mRNAs/miRNAs will be validated as potential biomarkers using clinical specimens from normal and osteoporosis patients.
Osteosarcoma cell lines were cultured in estrogen-stripped medium for 3 days and then treated with 1 nM of Estradiol for 48 hours. The results demonstrated a variation between the proliferations of cells affected by Estradiol. RNA was isolated from cultured cells and libraries were constructed for sequencing. Differential expression analysis revealed that Estradiol treatment in Mg-63 significantly upregulated the expression of 131 genes and significantly downregulated the expression of 73 genes by >2 fold changes when compared to non-treated controls ($p<0.05$). RNA was also used for microarray profiling to study the populations of microRNAs differently expressed between the treated cells and non-treated controls.
Future work is aimed at validating the identified up and down differentially expressed genes using Mg-63 cell line and then clinical specimens by qRT-PCR. This research will advance our knowledge and help us to understand mechanisms underlying osteoporosis. It will also generate a potential strategy to develop innovative diagnostic and therapeutic applications in the future to improve patients' healthcare.

P053

Delivery of pro-angiogenic and osteogenic genes to human skeletal and endothelial populations to augment bone repair

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Non-union of bone fractures arises when there is a failure of blood vessels to regenerate, thereby blocking the self-repair mechanisms of bone. Current surgical treatments are invasive, utilising bone grafts and metal plate fixation, while doing little to directly modulate angiogenesis. An attractive approach entails the use of injectable cell scaffolds augmented with vectors capable of delivering genes associated with increased angiogenesis and osteogenesis. The aims of this study were to increase the expression of pro-angiogenic and osteogenic genes in skeletal and endothelial cells to increase proliferation and function.

MG63 osteoblastic and EAhy926 human endothelial cell lines, primary human umbilical vein endothelial cells (HUVECs), and STRO+ skeletal stem cells (SSCs) were transfected with BMP2, HIF1a or VEGF transgenes using either plasmid or lentiviral vectors or lipofectamine delivery of RNA . Changes in gene expression were quantified by SYBR green real time PCR. DNA was quantified with the PicoGreen assay as a measure for cell proliferation.

Transfection of MG63 cells with plasmids for BMP2 and BMP2-P21 increased BMP2 gene expression at 48 hours by ~300-fold ($P<0.01$) and ~400-fold ($P<0.0001$), respectively, with no effect on proliferation. Transfection of MG63 cells with lentivirus had no effect on gene expression at 72 hours, however proliferation was significantly increased by BMP2 ($P<0.05$), VEGF-P21 ($P<0.0001$), and αVEGF ($P<0.05$). Similarly, lentiviral transfection had negligible effect on gene expression in EAhy926 cells after 72 hours, however VEGF-P21 significantly increased proliferation ($P<0.05$). HUVECs did not respond to lentiviral transfection, although recombinant VEGF increased proliferation ($P<0.01$). Transfection of enriched human skeletal stem cell (STRO+) populations with BMP2-P21 lentivirus significantly increased proliferation ($P<0.01$). On-going experiments indicate increased BMP2 gene expression and proliferation in both MG63 cells and STRO+ SSCs following BMP2 and BMP2-P21 RNA transfection.

These studies demonstrate the up-regulation of genes associated with angiogenesis and osteogenesis can be successfully transfected into bone and endothelial cells using plasmid, lentiviral and critically RNA to increase proliferation. Current studies are focussed on evaluation and validation within in vitro and ex vivo models prior to use in in vivo systems.

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P054

Spinal osteoarthropathy in alkaptonuria patients monitored by^{18}F -NaF PET/CT

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Alkaptonuria (AKU) arises from a genetic deficiency of HGD an enzyme involved in tyrosine metabolism. AKU is characterised by high circulating homogentisic acid (HGA) some of which is deposited as ochronotic pigment in connective tissues namely cartilage, leading to multisystemic damage dominated by premature severe osteoarthropathy. Ochronotic arthropathy begins in the spinal column with symptoms similar to osteoarthritis that present around the third to fourth decade of life.

Pathological changes in the spine as a result of pigmentation can be imaged using fluorine-18 labelled sodium fluoride positron emission tomography integrated with computer tomography (^{18}F-NaF PET/CT). This imaging modality allows quantitative assessment of focal bone remodelling by measuring the uptake of F18 into the hydroxyapatite crystal of bone and calcified cartilage.

^{18}F-NaF PET/CT scans of 10 female AKU (age range 52-72) and 10 female control (age range 43-84) patients were analysed using Hermes hybrid viewer. The standardised uptake value (SUV- mathematically derived ratio of tissue radioactivity in a ROI and the decay corrected injected dose per kilogram of the patient's body weight) was calculated for all lumbar and thoracic vertebrae and intervertebral discs (IVD's). The SUV was obtained at the center of each vertebral body and IVD's. Statistically there was no significant difference (P=0.43) between the SUV of AKU vertebrae (mean SUV=7.58 SD=1.3) and control vertebrae (mean SUV=7.58 SD=0.63) suggesting that generalised rates of bone formation in the spine are almost normal in AKU patients. When comparing the SUV's for AKU and control IVD's there was a statistically significant difference (P=0.009) between both thoracic and lumbar discs where AKU discs had a much higher SUV mean of 12.01 (SD=1.8) compared to control discs that had a lower SUV mean of 3.81 (SD=1.26). Multilevel IVD space narrowing associated with calcifications was evident in the AKU group as well as osteophyte formation. It is proposed that increasing HGA deposition within the IVD's leads to calcification composed of apatite crystals in the discs resulting in high uptake of F18. This quantitative measure of spine involvement could be useful in monitoring the efficacy of potential therapies to treat AKU and other osteoarthropathies.

P055

The relationship between the Fine Structure Analysis magnetic resonance imaging (MRI) technique and trabecular number (Tb.N) measured by micro computed tomography (microCT) in cadaveric vertebrae

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Objectives
Detailed information on bone microarchitecture at central sites that is comparable to high resolution CT but which can be obtained in vivo without the need for ionizing radiation is needed clinically. MRI has previously been applied for this purpose however there can be poor resolution and low signal-to-noise ratio at central sites. A novel MRI based Fine Structure Analysis technique (FSA developed commercially as fineSA®) which can deliver a high resolution spatial frequency spectrum representative of repetitive structure within the region of interest (ROI) at both central and peripheral sites was applied to human cadaveric vertebrae and compared to trabecular number (Tb.N) obtained from micro-CT (µCT) analysis at the same location.

Methods
Ten vertebral bodies (VB) (T7-L5) from two cadaveric human spines were individually imaged by both µCT and MRI. µCT scanning was performed with an isotropic voxel size of 37 µm (Scanco USA Inc. Wayne, PA), and FSA MRI data acquisition was performed in two regions of the vertebrae (anterior and central section) on a Siemens TrioTim 3.0T scanner. Rigid-body image registration was used to ensure analysis of the same location in the MRI and the µCT images. Linear support vector regression (SVR) on features from the resulting FSA spectra against target values of Tb.N obtained from µCT data produced a FSA metric.

Results
Strong and statistically significant correlations were found between the FSA metric and Tb.N (Pearson correlation coefficient $R=0.889$, $p<0.05$).

Conclusion
The close relationship between the information provided by FSA and Tb.N indicate the utility of MRI-based FSA for extracting a measure strongly correlated to Tb.N for in vivo applications such as the determination of osteoporosis and potential fracture risk.

P056

Differences in spine shape in adults entering old age: relationship with sex, body size and BMD

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Objectives: Like hip joint shape, which has been identified as a potential imaging biomarker for osteoporosis and osteoarthritis, individual spine shape is also diverse. However, its role in health and disease has not been investigated. This pilot study characterised spine shape in adults from the Medical Research Council National Survey of Health and Development (NSHD) and explored variations in relation to sex, bone mineral density (BMD) and anthropometry.
Methods: We analysed data from 200 individuals in the NSHD birth cohort, including height, weight, body mass index (BMI), spine BMD and lateral dual-energy x-ray absorptiometry (DXA) images taken at 60-64 years. A statistical shape model (SSM) was used to quantify variation in sagittal spine shape (5th lumbar - 10th thoracic vertebrae). SSM identified independent modes of variation in shape with mode scores quantifying deviation from the mean. Modes 1-10 (describing 88% of variation) were chosen for analysis. Sex- differences were evaluated by the Mann-Whitney test and Spearman's rank correlation used to explore associations between shape modes and potential predictors.
Results: The sample contained 90 men and 110 women aged 63.6 (62.8 - 64.2) years, (median [interquartile range]), with a median BMI of 27 kg/m2 (interquartile range 24.5 - 30.5). Men had more evenly distributed curvatures than women ($P<0.001$) and wider vertebral bodies ($P<0.0001$), while women were more likely to have a snaking curvature. Vertebral width increased caudally, together with narrower L4/L5 disc space, in women ($P<0.0001$). Narrower thoracic vertebrae ($P<0.01$) and a flatter curve ($P<0.01$) were seen in men. Correlations with height, weight and BMI varied by sex ($P<0.05$). In women, greater spine BMD was related to curvatures with distinct thoracic kyphosis and lumbar lordosis whilst in men an overall even curvature was related to greater spine BMD.
Conclusion: Vertebral width and distribution of spinal curvature, not overall curvature, differed between men and women in this sample of early old-age adults. Associations between spine shape, BMD and anthropometric measures varied by sex. These pilot data will enable future studies of associations between spinal morphology and musculoskeletal morbidity.

P057

Novel Silane Surface Modification Increases Expression of Osteocyte Markers in Primary Human Osteoblast-Like Cells under Standard Culture Conditions

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INTRODUCTION

Amine (-NH_2) functionalisation can be utilised to enhance the osteogenicity of a wide range of biomaterials. In this work primary human osteoblast-like cells were isolated from human bone and seeded onto a range of -NH_2-modified glass substrates, in order to determine if the osteogenicity observed in mesenchymal stem cells cultured on amine-modified surfaces was transferable to this more mature cell.

EXPERIMENTAL METHODS

A range of -NH_2-silane modified substrates were fabricated and comprehensively characterised. Cells were seeded onto modified materials (50,000 cells per material) and incubated in basic DMEM media with 5% FCS with no exogenous growth factors, for 7, 14 and 28 days. Samples were stained using Von Kossa's stain for mineralisation. Cell-material interactions and formation of matrix was qualitatively assessed using SEM (Leo/Zeiss 1550). Size and number of nodules were recorded. Quantitative evaluation of the mRNA expression of collagen I, osteopontin, osteocalcin, osteonectin, Cbfa1 and sclerostin was performed using real time polymerase chain reaction (qRT-PCR). All targets were normalised to b-actin and up-regulation of genes of interest calculated using the DDCt method. For all measurements of nodule size and qRT-PCR expression, a total of 4 repeats were carried out and differences in expression determined by ANOVA.

RESULTS AND DISCUSSION

Bone nodules were formed on amine modified substrates with significantly smaller peak heights (sub 20nm) compared with other modified substrates after 7 days. In addition, the expression of sclerostin ($p=<0.01$) was significant on the sub 20nm, a marker of osteocyte phenotype, was present on the materials with sub 20nm peak heights after 7 days, but not present on the -NH_2-modified substrates with larger peak height features.

CONCLUSION

Amine modifications produced cell-contacting materials that are osteoconductive. Subtle changes in surface chemistry and topography resulted in vastly different responses from the osteoblast-like cells, ultimately being an initiator of osteocytic markers.

P058

Investigation into the biomechanical properties of cancellous bone preserved using Thiel's fixation method

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Thiel's method is an anatomical fixation technique that is shown to have a combination of traditional durability of tissues but combined with realistic flexibility, particularly in the musculoskeletal system. Thiel's method is particularly suited to clinical applications where life-like model validation and biomechanical testing of cadaveric tissue is required. Unlike formalin-fixation, Thiel's fluid contains boric acid which partially denatures the collagen in muscles and tendons, resulting in a more flexible anatomical specimen. The aim of this study is to biomechanically test sections of Thiel preserved cancellous bone relating to the head and neck of human femora and to obtain its Young's modulus and yield strength. Cancellous bone from left and right proximal femora was obtained from two human cadavers - one preserved using traditional formalin-fixation and the other using Thiel's method. Left and right femora were harvested and sectioned into 12 equal segments, from proximal to distal. Proximal sections were used in this study (head, neck and trochanter's of each femur). Cancellous bone was excised and immersed in 10% SDS solution for 48 hours, 4°C to remove excess bone marrow, then repeatedly washed in 10% PBS solution. 10mm3 segments were cut (n=25 for the Thiel, n=21 for formalin) and subjected to mechanical testing using a compress to yield protocol with 2500N load cell. Initially, yield strength (MPa) and modulus (MPa) were significantly correlated in both samples [Formalin = 0.91 (<0.001); Thiel = 0.718 (<0.0038)] and RMA regression analysis revealed significant differences in the scaling of Young's modulus with stress at yield (<0.001) between Thiel and Formalin fixed specimens. However, as data was further collected, the correlation coefficients decreased [(Thiel = 0.439 (<0.009) and Formalin = 0.761 (<0.001)] which indicated that the difference in sampling may be due to human variation. To rule out human variation with regard to mechanical properties of cancellous bone, samples have been obtained from the same individual and subjected to Thiel and Formalin fixation separately. The biomechanical properties of Thiel-fixed and Formalin-fixed cancellous bone could have future implications in the biomechanical applications in the use of Thiel preserved cadavers where a life-like model validation is required.

P059

Old bones, new structures - detection of adipocyte templated excrescences in aged human bone

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Introduction
We have previously shown trabecular bony excrescences, apparently made and moulded by adipocytes in pathological bone samples from OA and alkaptonuria patients. There is a question whether these distinctive microanatomical structures are indicative of abnormal activity or osteoarthropathies, possibly secondary to altered mechanical loading or other aberrant signalling, or whether they may also be found in normal, non-pathological aged bone.
Methods
Proximal tibia were obtained from 3 cadavers with no clinical history of bone or joint pathology. The mean age was 88.3 years. Coronal sections were cut from the tibia and tissues were decalcified and processed for routine histology. Sections were either left unstained or stained with hematoxylin and eosin visualised using brightfield and polarized light microscopy as well as subject to autofluorescent examination. Numbers of excrescences were counted from each block of tissue and a map of their distribution plotted.
Results
Standard histological examination showed excrescences present in all 3 cadaveric samples. The average number of excrescences seen in each section (~2cmx1cm) was 2.7 in the epiphyseal region, 0.2 in the metaphysis and 3.5 in the diaphysis. The excrescences demonstrated poor integration with the existing trabecular bone domain and many of the excrescences showed continuity with the reticular collagen network that branched through the marrow cavity. Despite poor integration with the existing network of trabeculae a number of excrescences showed mature lamellar bone structure when viewed under polarised light and some also contained osteocyte lacunae. The marrow cavity surface of the excrescences showed morphology that was analogous with adipocyte morphology; a small number of adipocytes were seen on these surfaces and engulfed within the reticular collagen network extending from the excrescences.
Discussion
This study shows excrescences in "healthy" aged bone in epiphysis, metaphysis and diaphysis, suggesting that they have a global distribution within the bone with most abundance in the epiphysis and diaphysis. Further examination of age series of normal healthy tissues for excrescences and their relationship with adipocytes is warranted.

P060

Analysis of osteocyte density in mice using µCT

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Osteocytes are major local regulators of bone turnover. The density and distribution of osteocytes (Ocy) is therefore likely to affect bone turnover. Advances in micro-computed tomography (µCT) have enabled visualisation of details down to sub-micron levels. Ostecyte lacunae have a diameter of around 10 µm, and should therefore be detectable using µCT. Here, we describe a method for visualising osteocyte lacunae in mouse bone, and a comparison of wild type and neuronal nitric oxide synthase knockout (nNOS KO) mice, which are characterised by high bone volume and low bone turnover.
The left femurs of four 3-month-old female WT and nNOS KO mice were fixed overnight in buffered formalin and stored in 70% ethanol. The distal femurs were scanned at using a Skyscan 1272 desktop µCT system (50 kV, 100 or 200 µA, 0.5mm Al filter, 0.1° rotation step size), 3D image stacks reconstructed using NRecon, and bone tissue and osteocyte lacunae identified by image analysis using CTAn.
Initial scans performed at 3 µm pixel size failed to resolve the lacunae. Subsequent scans at 1.5 µm successfully resolved the osteocyte lacunae, however, at full X-Ray current (200 µA) the sharpness of the images was not optimal. Reduction of the current to 100 µA, which should reduce x-ray spot size, resulted in improved image quality. Sharpness was further improved by correcting for x-ray spot drift in NRecon. Bone tissue was identified by a simple threshold procedure. To identify osteocyte lacunae, image noise was reduced by median filtering, and edge contrast enhanced using an unsharp-mask filter. The lacunae were identified using a local contrast threshold algorithm. The Ocy.N was $52 \times 10^3 \pm 6 \times 10^3$ per mm^3 for WT and $43.3 \times 10^3 \pm 11 \times 10^3$ per mm^3 for the KO samples. The Ocy.volume/BV was 1.7±0.3% for WT and 1.4±0.4% for KO samples. Neither of these differences, however, reached statistical significance ($p>0.05$).
In conclusion, osteocyte lacunae can be identified by µCT, and osteocyte density measured. The results suggest a decreased osteocyte density in the nNOS KO, which may relate to the low bone turnover in these mice. However this result was not significant, possibly due to low replicate number.

P061

An audit of the recognition and management of low alkaline phosphatase levels in children

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Objective: Low serum alkaline phosphatase (ALP) is the hallmark of hypophosphatasia. There is evidence that it is often unrecognised by clinicians leading to a delay in diagnosis, inappropriate treatment and, in some cases, harm. Using the standard that an abnormal result should be recognised by the clinician, and the potential cause and need for further investigation documented in the medical records, we conducted an audit of our practice at the Royal National Orthopaedic Hospital.

Methods: Using the age specific reference ranges published by Pathology Harmony, the biochemistry database was searched to identify patients aged less than 18 years with an abnormally low ALP. The medical records of those identified were reviewed to find if the abnormal result was recognised, the medical history and further investigations performed.

Results: A search of 3,031 ALP assays measured over 3 years identified 71 abnormal results and 28 patients with a persistently low ALP. None of the medical records showed any recognition of the abnormal result, consideration of its cause or plan for further investigation.

Conclusions: Our results correspond to existing studies and show that clinicians frequently miss patients with an abnormally low ALP. This omission has and will continue to lead to undiagnosed cases of hypophosphatasia. This is of crucial importance for the paediatric population as a specific treatment is licensed and available and we are implementing a local guideline for the investigation of children with a low ALP. It is important that the awareness of the potential significance of low ALP values is increased and that ALP values are reported using an age adjusted lower limit of normal such as those produced by Pathology Harmony.

P062

Fibula adaptation to regular exercise indicates new mechanoadaptive behaviours of bone

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Fibula mediolateral bending strength is lower in the mid-proximal and mid-distal shaft than other regions. In disuse caused by long-term spinal cord injury this profile is flattened, suggesting a relaxing of this site-specific adaptation to habitual load. In addition, disuse-related bone mass loss and changes in bone geometry are much less pronounced than in the neighbouring tibia, which cannot be explained on the basis of differences in bone size or habitual loading. However, fibula adaptation throughout its length in response to exercise has not been completed, with previous studies examining only a single mid-shaft site.

Therefore in order to examine effects of regular exercise on the fibula throughout its length, peripheral quantitative computed tomography (pQCT) scans at 5% increments from 5-90% distal-proximal tibia length in 10 sedentary men (age 31.9±3.1y) and 10 trained male endurance runners (age 33.9±3.2y) were examined. Tibia data from these scans have previously been reported (Feldman et al., 2012). Group differences at each site were examined using factorial ANOVA, with results considered significant where $P<0.05$. Mediolateral bending strength (assessed by Bone Strength Index, BSIy) was lower in runners than sedentary men in mid-distal (10-45% sites, $P<0.001$) and mid-proximal (60-75% sites, $P=0.01$) fibula. Anterior-posterior bending and torsional BSIs were also lower in the distal fibula of runners, as was mid-proximal fibula cortical bone mass which is an indicator of compressive strength (all $P<0.05$). These results are in contrast to greater values observed for each of these measures at multiple tibia sites in the same scans, as reported previously.

These results suggest that regular exercise exaggerates the regional variation observed in fibula bone strength throughout its length. Negative effects of exercise on the fibula are in contrast to those observed in the other long bones in humans, and do not fit with the established Mechanostat theory. Together with other recent examinations of fibula structure, these observations suggest new site-specific behaviours of the adaptive processes governing limb bone structure.

Reference:
FELDMAN, S, CAPOZZA, RF, MORTARINO, PA, REINA, PS, FERRETTI, JL, RITTWEGER, J & COINTRY, GR 2012. Site and sex effects on tibia structure in distance runners and untrained people. MSSE, 44, 1580-8.

P063

MicroRNA miR-1231 inhibits expression of sclerostin in TE85 and SaoS-2

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MicroRNAs (miRNA) are negative regulators of gene expression through the inhibition of the translation of messenger RNA (mRNA). miRNAs are involved in major cellular functions and have been implicated in various human diseases but their role in bone and in the aetiology of metabolic bone diseases is unclear. The aim of this study was to investigate the involvement of miR-1231 in the regulation of sclerostin, a major controller of bone formation. We investigated the expression of miR-1231 in two human osteosarcoma cell lines representing different stages of osteoblast differentiation: TE-85 representing the intermediate stage of differentiation and SaOS-2, representing the most mature stage. Cells were transfected with scrambled miRNA (as negative control), miR-1231 mimic and miR-1231 antagomir using Lipofectamine 2000 in serum free media. After 6 hours of incubation, serum free media was replaced with media containing 10% bovine calf serum. Non-transfected cells were also used as control. Total RNA was extracted after 48 hours using miRNeasy Mini Kit (Qiagen). In cells that were transfected with miR-1231 mimic, real-time PCR results show a dramatic increase in miRNA-1231 expression in both TE85 and SaoS-2 (4.12±0.93 and 2.24±0.47 relative to RNU6_2, $p<0.05$) indicating the miRNA-1231 transfection was efficient. TE85 and SaoS-2 that were transfected with scrambled miRNA demonstrated no significant change in the expression of miR-1231 compared to the non-transfected cells as the scrambled miRNA does not bind to any known mammalian mRNA. In contrast, TE85 and SaoS-2 that were transfected with miR-1231 antagomir shows a small reduction in miR-1231 expression. Interestingly, our results show a significant decrease in sclerostin gene expression (relative to β-actin) in both TE85 (0.54±0.06, $p<0.05$) and SaoS-2 (0.55±0.08, $p<0.05$) in cells that were transfected with miRNA-1231 mimic. This result suggests that miR-1231 may inhibit the translation of sclerostin mRNA in osteoblastic cells. Further experiments are required to verify whether miR-1231 could be considered as a candidate for a new class of therapeutic drug to prevent bone loss.

P064

An anatomical investigation of a cadaver displaying characteristics of both enchondromas and osteochondromas

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Ollier's disease is a rare condition with a prevalence of 1/100,000. It is characterised by multiple enchondromas (benign cartilaginous neoplasms) manifesting asymmetrically in areas close to the metaphysis. This idiopathic disease results in skeletal dysplasia with the potential for malignant change. Hereditary multiple exostoses is a condition that results in growths on the surface of the bone and has a prevalence of 1/50,000. Like Ollier's disease, this condition is caused by abnormal cell growth of the metaphysis.

A male donor who had been diagnosed with Ollier's disease in life was bequeathed for anatomical examination to the University of Liverpool. After undergoing x-ray, CT and MRI scans it was apparent that the donor suffered from osteochondromas in addition to the enchondromas present in Ollier's disease. Furthermore, unusually for Ollier's disease, there were no large enchondromas present in the phalanges of the hand. Growths were observed in the long bones of the skeleton; specifically in the phalanges of the feet, the metatarsals, the fibular, femur, radius and humerus bilaterally.

On dissection, the influence the exostoses had on the surrounding tissue was apparent; with growths causing muscle deformity and impingement of nearby nerves. One notable observation was the insertion of muscle tendons into the larger tumours, suggesting a link between muscle traction and growth size.

The exposed exostoses showed a classic cartilaginous cap with underlying bone. Notably, The unusual right radial head was initially thought to be an exostosis. Through dissection, it presents as an enchondroma in the diaphysis disrupting the radial head articulation with the humerus. Thus a new joint had formed with the radial tuberosity and the capitulum of the humerus that appears to have incorporated the biceps brachii tendon into the joint cavity.

This works constitutes an ongoing investigation that aims to show the gross and micro anatomy of these conditions and will attempt to determine if a link can be made between the two conditions given their similarities in growth plate dysfunction.

P065

BL-1249 is a K^+ channel opener and inhibits induction of the intermediate early gene *c-fos* in the SaOS-2 osteosarcoma-derived cell line

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Potassium (K^+) channel modulation has the potential to regulate the activity of bone-forming osteoblasts. We have investigated the effects of the K^+ channel opener BL-1249 on the SaOS-2 cell line, which is derived from a human osteosarcoma. BL-1249 (10 mM) hyperpolarised SaOS-2 cells and activated a K^+ current. BL-1249 activation of current was prevented by pre-incubation with penitrem A (200 nM) and tetraethylammonium ions (TEA^+, 2 mM), both inhibitors of the large conductance Ca^{2+}- and voltage-activated K^+ (BK_{Ca}) channel. At concentrations of 100µM BL-1249 caused large cell death to the SaOS-2 cells as well as other osteosarcoma cell lines MG-63 and Te-85. TEA was able to prevent the decrease in cell numbers. Interestingly this apoptotic effect was not observed in primary human osteoblasts.

Effects of BL-1249 on induction of the intermediate-early gene *c-fos* were studied using a reporter cell line. BL-1249 (10 mM) inhibited basal activity of *c-fos*. BL-1249 also inhibited the induction of *c-fos* by PTH (0.5 nM), with an IC_{50} of 16.6 mM, but not induction by foetal calf serum. Activation of *c-fos* by PTH occurs via adenyl cyclase, elevation of cAMP, and subsequent activation of cAMP-dependent protein kinase (PKA). Direct activation of adenyl cyclase by forskolin (10 mM) caused induction of *c-fos* and this was inhibited by BL-1249 (IC_{50}=6.9 mM). The role of membrane potential hyperpolarisation in inhibition of *c-fos* by BL-1249 was studied. Elevating extracellular K^+ to 40 mM to clamp the membrane potential did not alter BL-1249 inhibition of *c-fos*. The BK_{Ca} channel activator NS-1619 (10 mM) did not modify induction of *c-fos* by PTH. In conclusion, we have identified two novel pharmacological actions of BL-1249: BK_{Ca} channel activation and inhibition of the induction of *c-fos*. However, membrane potential changes associated with K^+ channel activation do not appear to be central to the inhibition of *c-fos* levels seen with this compound in SaOS-2 cells.

P066

Early Detection of Osteogenic Changes in Normal Human Osteoblasts by Raman Spectroscopy

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Objective
Raman spectroscopy is a label free technique which has proved to be a useful tool for detecting early biochemical changes in cells. It is a highly sensitive technique which does not need any exogenous labelling and is also non-destructive, providing molecular information about individual living cells. Raman has been used successfully in various biomedical applications and its clinical diagnostic potential has been demonstrated for various diseases. The main objective of this study was to assess whether Raman Spectroscopy could be used to detect early stages of human osteoblast (HOB) differentiation.

Methods
HOBs were extracted from bone explants collected from normal trabecular and cortical bone sites (proximal humerus). Cells were plated on Quartz coverslips and cultured for up to 21 days before fixing with 4% paraformaldehyde. 30 nuclear spectra were collected per sample using the Renishaw® inVia Raman microscope with a 532 nm laser and a Leica 63x (NA:1.2) water immersion objective. Parallel studies were undertaken assaying cells for alkaline phosphatase (ALP) activity and gene expression during the 21 day culture period.

Results
Levels of ALP eluted from culturing trabecular HOBs reached levels of significance following 7 days of culture ($p=0.045$). In contrast, analysis of collected spectra from day 3 and day 5 of HOB culture revealed significant increases in both amorphous calcium phosphate ($p=0.0012$) and carbonated apatite ($p=0.014$) between these time points. As predicted in early stages of osteogenesis the proline peak associated with collagen content ($p= 0.009$) was significantly altered between 3 and 5 days. Thus it appears that Raman spectroscopy can provide enhanced sensitivity in detecting early stages of HOB differentiation in vitro allowing for significant changes in phosphate to be detected between days 3 and 5.

Conclusion
We have previously published that HOBs grown from trabecular, subchondral and cortical explants from osteoporotic patients exhibit reduced levels of ALP versus osteoarthritic1. Our future studies will now use Raman to establish how early osteogenic spectra are altered in osteoblasts grown from diseased explants.

1. Shah M., et al. European Cells and Materials (2015), 29: 155- 176.

P067

Identification of regional diversity in blood vessel structures within murine cortical bone

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Objectives
In young individuals cortical porosity which includes osteocyte lacunae and blood vessel canals is thought to be homogeneously distributed. But with age porosity can reach up to 20% in 80 year olds and age-related defects may have implications for biomechanics and fracture resistance.
To date the global and regional distribution of cortical blood vessel canals with age in the murine tibia is disputed and will be the focus of this study.

Methods
The fibula-tibia junction from 15 week and 10 month old C57BL6 female mice were scanned using synchrotron-radiation computed tomography at the Swiss Light Source at a resolution of 0.65 µm. Reconstructed datasets were processed and analysed using commercial software to detect mineralised tissue, extract bone porosity and separate blood vessel canals from osteocyte lacunae. A 3-D distance transformation computed distances between osteocyte lacunae and the nearest available canal or bone surface.

Results
Global measurements of porosity did not show any significant differences in % canal volume with age. When cortices were separated into anterior and posterior regions we identified a vertical intracortical canal in the posterior region. Osteocyte distance analyses in the posterior region showed osteocyte lacunae present in this region up to 125.562 ± 7.111 µm away from nearest bone surface or intracortical canal. This is in contrast to the anterior region where osteocyte lacunae exist up to 102.8 ±4.73 µm from any bone surface and appear not to be reliant on intracortical canals for survival. In the anterior region % canal volume was not significantly altered with age ($p=0.107$) however, within the posterior region 10 month old animals had a % canal volume which was significantly less than 15 week mice (44.8% reduction $p=0.012$). Osteocyte lacunar density was also less in posterior regions (5.267 % reduction $p=0.0002$) but was unaltered with age.

Conclusions
In contrast to the predicted regularity of vascular porosity within the tibia fibula junction we have found distinct vascular structures within the anterior and posterior regions which appear to be differentially regulated with age.

P068

Bone loading by programmed muscle activity in the rat

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The responses of bone and joints to programmes of external loading has been an important part of the experimental study of skeletal adaptation. New developments in implantable pulse generators made in collaboration with the Medical University of Vienna now allow us to elicit bone loading protocols by programmed activation of skeletal muscles.
We have used FE models to predict the stress distribution within the tibia in response to common peroneal activation causing tetanic contraction of the tibilalis anterior muscle. The FE model predicted an area of maximum strain in the anterodistal part of the tibia. We delivered repetitive activation of the dorsiflexor muscles of the left hind limb via the common peroneal nerve over a period of 4 weeks. The induced contractions were very short, only 200 ms, and repeated every 30s. This is equivalent to activation for just 9.6 minutes per day.
We identified by micro CT that the area in which the stress due to contraction was predicted to be maximal showed bone growth with a significant increase in cortical thickness of about 300 microns. Targeted histological investigation of this area confirmed a region of primary osteon formation with a lower elastic modulus than the established bone. (Vickerton et al 2014)
The FE analysis of activation of the dorsiflexors confirmed a bending force on the tibia, resulting in the area of maximum strain in the anterodistal region. We have performed recent experiments in which the plantarflexors have been activated concomitantly with the dorsiflexors. We anticipate that the mechanical influence on the bone will be more nearly a compressive loading. We will use the same methods to investigate the response of the tibia to this new programmed loading regime.
Proc Biol Sci. 2014 Aug 7;281(1788):20140786. doi: 10.1098/rspb.2014.0786.
Morphological and histological adaptation of muscle and bone to loading induced
by repetitive activation of muscle.
Vickerton P, Jarvis JC, Gallagher JA, Akhtar R, Sutherland H, Jeffery N.

P069

Developing a more representative model of physiological and pathophysiological osteochondral cyclic loading

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Progressive hyaline cartilage and bone degeneration within the knee often leads to focal lesions, and eventually osteoarthritis (OA), a major clinical issue. Once initiated, progression of OA is linked to changes in biomechanical measurements including the external knee adduction moment (EKAM), a surrogate measure of medial knee compartment loading. A novel in vitro osteochondral explant compression loading model has been developed using EKAMs extracted from healthy or late-stage OA subjects during dynamic gait analysis, to better understand how biomechanical changes due to OA progression may lead to changes in the tissue response (peak deformation/stress relaxation) to cyclic load.

Dynamic EKAM waveforms for healthy and late-stage OA subjects were measured and extracted using a 9-camera motion capture system (Qualisys, UK) and force plates (Bertec Corp, USA). The main discrepancies were used to create two possible extremes of waveform (NL = normal, OA = osteoarthritic), to allow distinction of the two groups. Cylindrical osteochondral explants (n=6) were extracted from repeatable healthy regions of knee replacement surgery waste tissue using an electric drill with an 8mm diameter core diamond drill bit. Explants were randomly separated into 2 groups subjected to either 1800 cycles of NL or OA waveforms at 1Hz with a peak load of 2.5MPa. The application of cyclic compression load and measurement of peak explant deformation was made every 200 cycles using a BOSE ElectroForce 3200® (Bose Corporation®).

Results: Higher average peak tissue deformation (1.5:1 at 1800th cycle) was measured in OA loaded explants compared to NL groups at every load cycle milestone (every 200 cycles), but a repeated measures ANOVA revealed no statistical differences ($p<0.05$) between the two groups for the duration of the loading. During cyclic loading, OA waveform loaded tissue demonstrated a longer stress relaxation time compared to OA waveform.

The results from this methodology suggest that cyclic loading with EKAM waveforms typical of osteoarthritic gait in comparison to healthy gait may have a relatively damaging influence on mechanical response of viable osteochondral explants, however there is no statistical evidence to back this result due to high variability. Higher explant numbers are required to further conclude these results.

P070

Tissue distribution of mRNA expression of enzymes involved in the tyrosine metabolic pathway in *Hgd*$^{-/-}$ mice with the osteoarthopathy of alkaptonuria.

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Introduction

Alkaptonuria (AKU) is a rare genetic disorder of tyrosine metabolism caused by the lack of homogentisate 1,2-dioxygenase (HGD). The condition is characterised by excessive accumulation of homogentisic acid (HGA) in body tissues. This accumulation of HGA causes ochronosis, a deposition over time of melanin-like pigmented polymers in the extracellular matrices of connective tissues, especially cartilage, leading to severe degenerative osteoarthropathy. There are two new hypotheses for the production of this pigment, one that it is a result of enzymes, HGD and *p*-hydroxyphenylpyruvic acid (HPPD), located within specific tissue sites where ochronosis is observed, the other that tyrosine hydroxylase (TH) and tyrosinase (TYR) are involved in the polymerisation process of HGA.

Objective

To use the mouse genetic equivalent of human AKU (*Hgd*$^{-/-}$), to ascertain whether or not production of ochronotic pigment is a result of local tissue enzymes or as a result of excessive accumulation of HGA from the extracellular fluid.

Methods

6 BALB/c *Hgd*$^{-/-}$ mice (3 female and 3 male) were culled and tissues (brain, liver, kidney, bone, cartilage, muscle, spleen and eyes) harvested for RNA extraction to produce cDNA. Primers were custom designed for mouse HGD, HPPD, TH, TYR and β-actin. Analysis of mRNA expression was performed by QPCR.

Results

Significant amounts of HGD were detected in both the liver and kidney as expected, but none was detectable in the cartilage. HPPD was most abundant in the liver, with smaller amounts found in the kidney and trace amounts detected in the other tissues. Both results suggest that the ochronotic pigment found in AKU patient tissues, is a consequence of the enzymes, whether functional or non-functional, within the liver and possibly the kidney.

TH was expressed in the eyes and brain and TRY was found primarily in the eyes. It is clear that from both the distribution of TH and TYR, that these enzymes are not involved in the polymerisation of HGA to ochronotic pigment.

Conclusion

Findings suggest that the ochronotic pigment is a result of excessive accumulation of HGA in extracellular fluid rather than local production from enzymes within the affected tissues.

P071

A Metabolomics approach to changes associated with loss of cartilage in Alkaptonuria and Osteoarthritis: A Preliminary Study.

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Osteoarthritis (OA) is associated with destruction of cartilage and is widespread in Alkaptonuria (AKU), a serious genetioarthritic disease caused by a deficient enzyme, homogentisate 1,2-dioxygenase (HGD) and with morbidity caused by increased levels of homogentisic acid (2,5-dihydroxyphenylacetic acid, HGA) and resultant pigmentation particularly cartilagenous tissue. To investigate whether there are any similar metabolic processes involved by such loss of cartilage in idiopathic OA (iOA) and OA resulting from AKU (AKU-OA) a non-targeted metabolomics approach using UHPLC/QTOF MS was performed. Urine samples were analysed from two patient groups; one with well-established iOA and another AKU-OA. The spectra were compared with a control group.

Urine samples from patients (n=17) with iOA, patients (n=30) with AKU-OA and age-matched healthy volunteers (n=12), were analysed using reverse-phase UHPLC on an Agilent 1290 Infinity UHPLC system coupled to an Agilent 6550 Quadrupole Time-of-Flight mass spectrometer in positive and negative polarity Electrospray Ionisation.

The data were processed using a feature finding algorithm (*Molecular Feature Extractor).* This interrogates the 3-D data space extracting chromatographic peaks representing compounds and the associated MS spectra including isotopes, adducts and multimers. The extraction and data review were performed using *Mass Hunter Profinder* software for alignment and elimination of false positives/negatives. The resulting 'found' entities were compared across the three sample sets using *Mass Profiler Professional*.

Interrogation of the variance across all datasets using Principle Components Analysis showed clear separation between the metabolic profiles of AKU-OA patients compared with iOA patients and controls. However, a number of entities were identified as up or down regulated in both AKU and iOA compared with controls. Putative identities for these entities can be derived based on accurate mass and retention time using web-based databases (*e.g.*METLIN), supporting further investigation using a more-targeted metabolomics approach.

Metabolomic analysis identified a number of entities that were up or down-regulated in both idiopathic OA and AKU OA compared to healthy subjects. Although the compounds were not definitively identified via the use of reference standards, the data demonstrates the value of this approach in clinical metabolomics and offers the possibility of discovering multiple compounds associated with different forms of a disease.

P072

The effect of continuous and intermittent exercise on bone mineral density in postmenopausal women: a six-month randomised control trial

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Objectives
Postmenopausal women experience rapid declines in bone mineral density (BMD). High-impact exercise has been shown to reduce postmenopausal bone loss, with intermittent mechanical loading suggested to generate a greater BMD response than continuous mechanical loading. The aim of this study was to evaluate the effectiveness of both continuous and intermittent high-impact exercise interventions for reducing bone loss in postmenopausal women.
Methods
24 postmenopausal women (1-5 years) were randomly assigned to complete either 30 continuous (0.25 Hz) countermovement jumps (CTS; N = 8), 30 intermittent (0.07 Hz) countermovement jumps (INT; N = 8) or no exercise (CON; N = 8). CTS and INT groups trained three times per week for six-months, with 48 h separating each bout. BMD was quantified using DXA of the lumbar spine (L1-L4) and femoral neck. Raw or log-transformed data were analysed using Cohen's d (0-0.19 trivial, 0.2-0.59 small, 0.6-1.19 moderate) with uncertainty expressed as 95% confidence intervals, together with the chance (%) that the true effect was trivial, substantially positive or negative. BMD (g.cm-2) percentage changes are presented for within-group changes.
Results
There were trivial changes in lumbar spine BMD for CTS (-0.35% reduction; d = -0.02 [-0.13 to 0.10]; 99% likely trivial), INT (-1.7% reduction; d = -0.11 [-0.16 to -0.05]; 100% likely trivial) and CON (-0.4% reduction; d = -0.03 [-0.14 to 0.07]; 99% likely trivial). There were trivial changes in femoral neck BMD for CTS (-0.5% reduction; d = -0.05 [-0.28 to 0.18]; 90% likely trivial) and INT (-1.6% reduction; d = -0.13 [-0.21 to -0.06]; 97% likely trivial). However, there was a small reduction in femoral neck BMD for the CON group (-1.5% reduction; d = -0.24 [-0.45 to -0.03]; 67% likely small).
Conclusion
Both continuous and intermittent countermovement jumping prevented a small reduction in BMD at the femoral neck. Lumbar spine BMD was maintained across all groups.
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P073

Insertional anatomy of the Peroneus brevis tendon and its relevance in surgical management of the Jones Fracture

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Objectives: The fracture of the metaphyseal-diaphyseal junction at the base of the 5th metatarsal is known as Jones fracture. One of the surgical managements of the Jones fracture is plating. The aim of this study was to evaluate insertional footprint of the peroneus brevis tendon (PBT) and to define "safe zone" for placement of the plate.

Methods: Forty-one formalin fixed cadaveric feet were dissected to evaluate insertional footprint of the PBT. After isolation of the peroneus brevis muscle, dissection was carried out along the tendon up to its bony insertions. Subsequently, insertional footprints were identified on the bone surface and marked with ink. Photographs were taken after each step of the footprinting process. To assess the PBT footprint at the base of the 5th metatarsal, following features were evaluated: area of insertion (AOI) (mm^2), length (mm), width (mm), position and shape. To determine position of the PTB footprint, the most proximal aspect of the 4th and 5th metatarsal articulation was set as a reference point. A perpendicular line was drawn from that point and position of the PBT insertion at the base of the 5th metatarsal was distinguished, as positive when PBT footprint crossed the line and negative when PTB footprint was behind or on the line. All measurements were performed using Image J software.

Results: The mean AOI of the PBT at the base of the 5th metatarsal was 54.49 ±16.46 mm^2, the length 15.98 ± 5.11 mm and the width 4.69 ± 1.39 mm. Sixteen feet demonstrated to have positive type and twenty-five negative. Analysis of the shapes of the AOI at the base of the 5th metatarsal revealed four different types: kidney (n=12), diamond (n=9), oval (n=7) and crescent (n=13). Eleven (26.8%) specimens showed evidence of additional bony insertions.

Conclusion: This anatomical study evaluated variations and insertional footprint of the PBT. Findings of this study can be used to identify "safe zone", to prevent damage of the PBT in fixation of the proximal 5th metatarsal fractures.

P074

Autosomal Dominant High Bone Mass Syndrome presenting before skeletal maturity

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A 10-year-old participant in the Cambridge Baby Growth Study (CBGS) cohort was referred to the Addenbrooke's bone clinic after the CBGS team discovered an abnormality on his whole body DXA scan. His total body BMD was +4.4 standard deviations (SD) above the 10-year-old mean despite him being of unremarkable weight and height (+0.84 SDs). His only significant past history was pre-school speech impediment associated with 'blocked auditory canals', resolving without surgery. His mother had noted his proficiency during rugby matches, particularly in contact situations. High-resolution peripheral quantitative computed tomography (pQCT; Xtreme CT, Scanco) showed an increased bone surface area and average bone density in the radius and tibia, compared to local age-matched reference values.

At 45 y/o his mother also attended for total body DXA, yielding a Z score +3.1 despite normal BMI of 23.2 kg/m^2 (total body fat -0.3SD). T-scores for the right and left femur, and L2-4 were all above the mean (1.4, 1.8, and 2.4 respectively). Symmetrical painless bony prominences were noted on the dorsum of her hands (abnormalities also reported in a maternal aunt), with milder prominence bilaterally in malleoli, tibial plateaus, iliac crests, and elbows. Oral examination revealed tori mandibularii (<3mm diameter). She reported difficulty with swimming and buoyancy. Her two other children had normal bone density. There was no family history of fractures or bone pain. Bilateral X-ray imaging of the mother's hands and knees showed cortical thickening and increased trabecular density. Xtreme CT of the radius and tibia demonstrated increased trabecular bone volume:tissue volume ratio, with increased numbers and thickness of trabeculae, and increased peripheral trabecular bone.

These results confirm HBM syndrome in the index case and her son, with an autosomal dominant pattern of inheritance, although whole exome sequencing for common HBM variants was negative. Monitoring the trajectory of bone mineral density accrual in a pre-pubertal boy with HBM will provide insights into the natural history of this rare condition.

P075

Up the creek: truthful imaging in spinal osteoporosis?

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It is impossible for x-ray computed tomography (CT) or magnetic resonance imaging (MRI) to resolve fine structural details in calcified tissues in large, live bones. This can only be done ex vivo or post mortem. Imaging dead bone, it may be held, does not aid the live patient, but imaging live bone cannot reveal the true situation.

The basic principles of stereology demand the use of infinitely thin sections, such as polished surfaces of rock samples. But bone tissue is not a solid, and therefore it has to be embedded – usually in a resin. Backscattered electron SEM of flat block surfaces – best micro-milled, but second best polished – provides very thin electron optical sectioning of vast extent and images selectively the calcified tissues in bones. Earlier difficulties were: (1) negative electrostatic charging under the electron beam, solved, even for uncoated samples, by having the sample chamber at 'bad' vacuum, when positive gas ions eliminate the problem, and: (2) inability to see soft tissue histology, solved by iodine staining of the block surface.

In this study, computer controlled digital SEM allowed the stitching (montaging) of large numbers of fields to provide very high resolution re-imaging of entire mid-body, near-midline, vertical sections through PMMA embedded L2 vertebrae (38 male, 31 female, 70±15 years, European Union Concerted Action Biomed 1 'Assessment of bone quality in osteoporosis').

What we learn is how very little and skimpy may be the bone tissue within apparently intact vertebral bodies. Thus parts of cortices are regularly less than one tenth millimetre thickness and trabeculae so fine that they could not possibly be found with clinical imaging. Within and towards the end-plates, calcified cartilage, more densely mineralised than bone, is a major contributor to radio-density. Clinical imaging could never discriminate what is bone, find fine trabeculae or canals within them or show the arrangement of microcallus; all easily achieved with BSE-SEM. This method also shows that the earliest stages of trabecular microfracture healing may employ acellular high density mineralised infill in addition to woven bone. Studies in this field are truly up the creek without BSE-SEM.

P077

P2X7 Receptor status alters the osteoblast response to clinically relevant concentrations of metal ions following metal-on-metal hip replacement

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High failure rates of metal-on-metal hip replacements(MOMHR) highlighted the detrimental effects of prostheses derived cobalt(Co) and chromium(Cr). It has now become evident that all modular hip replacements release metal ions as a result of tribocorrosion at modular junctions. Previous studies by us and others have shown that these metal ions are detrimental to the survival and function of bone cells. P2X7 Receptor(P2X7R), an ATP-gated ion channel capable of forming nonselective membrane pores, is an important regulator of normal bone homeostasis. Recently, we have demonstrated that antagonising P2X7R reduces the uptake of Co^{2+} in human osteoblasts. In this study, we show that P2X7R status plays a role in the functional response of osteoblast-like cells to clinically relevant concentrations of metal ions.

Te85 osteoblast-like cell-line that do not express a functional P2X7R, were transfected with two naturally occurring isoforms of P2X7R - a full length P2X7RA, and a truncated isoform P2X7RB. On reaching confluency, the cells were treated with either Co^{2+}, Cr^{3+} or a combination of Co^{2+} and Cr^{3+} ions at clinically relevant concentrations of 50, 500 and 5000µg/L in osteogenic media containing 50µg/mL ascorbic acid and 10nM dexamethasone, with fresh treatments every 2-3 days. Inorganic phosphates were added for the last 3 days to promote mineralisation. Subsequently, the cultures were stained with Alizarin Red and percentage area of mineralisation quantified using ImageJ (https://imagej.nih.gov/ij/).

Co^{2+} treatment (5000µg/L) decreased the percentage mineralisation by 41% for naïve cells ($P<0.05$), 64% for P2X7RA cells ($P<0.0001$) and 19% for P2X7RB ($P<0.05$) compared to untreated controls. Cells expressing P2X7RA had 45% greater decrease in mineralisation compared to cells expressing P2X7RB ($P<0.05$). Cr^{3+} treatment at 5000µg/L reduced mineralisation by 24% for P2X7RA cells ($P<0.01$), whilst no effect was observed on naïve or P2X7RB cells. The Co^{2+}:Cr^{3+} combination treatment reduced mineralisation for all three cell lines at 5000ug/L ($P<0.0001$).

This data suggests that different isoforms of P2X7R elicit a different functional response in osteoblasts following metal ion exposure, particularly at concentrations equivalent to patient hip aspirates. This identifies P2X7R status as a potential novel marker to recognise patients at higher risks of hip replacement failures due to metal debris.

P078

P2X7RA or P2X7RB expression confers a growth advantage to Te85 osteosarcoma cells *in vitro.*

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Osteosarcoma is the most common type of primary bone cancer mainly affecting adolescents, it is a rare incurable disease with current treatments including chemotherapy and amputation. Survival statistics have remained constant for a number of years suggesting the need for new treatments. Purinergic signalling involves the binding of extracellular nucleotides including ATP to purinergic receptors and has been found to play a role in many cellular processes. Purinergic signalling acting on bone cells has been demonstrated with roles in differentiation, apoptosis, and bone remodelling. Additionally, purinergic receptors have been found across a variety of cancers with implications in tumour formation, progression and metastasis. High concentrations of ATP have been observed within the tumour microenvironment which could act on the P2X7 receptor, a type of purinergic receptor that acts as a ligand-gated ion channel and plays a role in cell behaviour, including increased cell proliferation. This receptor has been previously found on osteosarcoma tumours. In this study we provide evidence that expression of P2X7R isoforms modulate proliferation of osteosarcoma cells particularly under low serum conditions.

Te85 osteosarcoma cells, either naïve or previously transfected with the P2X7RA or P2X7RB isoform, were seeded at various cell densities (1.25, 2.5 and 5 x103). After 24 hours, the media was changed to either 10%, 2% or 0.5% FCS. Cell proliferation was assessed using an MTS assay at days 0,1,3,5 and 7. Statistical analysis was performed in Graphpad Prism® using One-way ANOVA with Tukeys post-hoc test and linear regression compared slopes of the growth curves.

At all serum concentrations and cell seeding densities, P2X7RA and P2X7RB had significantly increased growth rates compared to naïve cells ($P<0.0001$ for both), with no difference between the growth rates of P2X7RA and P2X7RB.
The naïve cells had a longer lag phase and caught up with the transfected cells towards day 5 and 7 under 10% serum conditions only.

These results suggest that P2X7RA and P2X7RB expression confers a growth advantage to Te85 osteosarcoma cells in low serum conditions which may contribute to tumour growth and invasiveness. Thus, P2X7R could be a potential new target for treating osteosarcoma.

P079

Collagen becomes ordered prior to mineral deposition

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Objectives
The mechanism of the mineralisation of collagen is not fully established. Osteoporosis and osteoarthritis are debilitating bone diseases that are associated with a change in bone composition and bone mineralisation. The study and diagnosis of bone diseases are largely based on X-ray technologies but these methods do not provide information on the component of bone and therefore do not fully characterise this complex material and its role in bone strength. Raman spectroscopy however may be used non-invasively, and transcutaneously, to provide an overall biochemical signature.
Turkey leg tendons (TLTs) are recognised as a model organ for studying distinct regions of mineralised and non-mineralised collagen. The aim of this study is to test the hypothesis that Raman spectroscopy can be used to identify differences in the collagen secondary structure between regions of young TLTs that will remain non-mineralised and those that become mineralised.
Methods
Extensor tendons from six ‘young' turkeys (11 weeks old; no mineralisation) and six ‘mature' turkeys (18 weeks of age; distinct mineralised sections) were collected and spectra were acquired along each tendon using a Renishaw inVia (Renishaw plc, Gloucestershire, UK) Raman microscope (830 nm excitation wavelength).
Results
Spectra across the transition zone of the mature TLTs and radiographs were used to confirm the presence/absence of mineral corresponding to the mature/young TLTs. Further Raman analysis revealed that as the phosphate (mineral) peak increased in height there was a change in the secondary structure of collagen, correlating to an increase in the Amide III : Amide I. Analysis of the young TLTs also revealed a change in Amide III : Amide I along the length of the tendon and at the predicted transition zones.
Conclusion
The data confirm the hypothesis and establish that collagen becomes more ordered prior to mineralisation. These results may help identify the mechanisms controlling mineralisation and contribute to new treatments of bone diseases, where mineralisation has been disrupted.

P080

Osteoclast Function is Modulated by Circulating Metal Ions from Patients Following Hip Resurfacing.

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We have previously observed an increase in total bone mineral density and reduced bone turnover (TRAP5b and osteocalcin) in patients with well-functioning metal-on-metal hip resurfacing (MOMHR). Here, we provide data to support the hypothesis that osteoclast function is altered in this patient population, and that this effect is transferrable through their serum.

Patients with well-functioning MOMHR (cases, n=18) at a median follow-up of 8 years were individually matched for gender, age and time-since-surgery to a low-exposure group consisting of patients with THA (controls, n=18). The monocyte fraction of patient peripheral blood was isolated and differentiated into osteoclasts on dentine wafers using RANKL and M-CSF supplemented media (osteoclastogenic media, OM). Cultures were monitored for the onset of resorption, at which point the cells were treated with OM, autologous serum or serum from matched MOMHR/THA donors, all supplemented with RANKL and M-CSF. At the end of the culture, cells were TRAP-stained and quantified using CellD Software Package, Olympus.

When cells were differentiated in standard osteoclastogenic media, the resorbing activity of osteoclasts derived from MOMHR patients was reduced 22% ($p<0.0079$) compared to THA. The resorbing activity of osteoclasts generated from MOMHR patients and differentiated in autologous serum was reduced 33% ($p<0.0001$), whilst matched THA serum caused a smaller reduction of 14% ($p<0.01$). When cells derived from THA patients were differentiated in autologous serum, the resorbing activity of osteoclasts was similarly reduced by 35% ($p<0.0001$), whilst the matched MOMHR serum also caused a reduction of 21% ($p<0.0001$).

This data suggests that prior exposure to higher circulating Co and Cr in patients with MOMHR reduces osteoclast activity, and that the detrimental effect on the functionality of mature osteoclasts is transferable through the serum. This has implications for systemic bone health of patients with MOMHR or modular taper junctions.

P081

Is earlier walking onset associated with greater hip bone strength in older men and women?

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Age at attainment of independent walking has a strong effect on bone strength in young children, but it is unknown whether associations persist into older age. To investigate these associations, age of independent walking was assessed by maternal questionnaire at two years of age, and hip bone strength was assessed by dual-energy X-ray absorptiometry (DXA) hip scans at 60-64 years in a large nationally representative British birth cohort study (MRC National Survey of Health and Development).

Total hip bone mineral density (BMD), bone mineral content (BMC) and bone area (BA) were measured, and femoral neck (FN) BMD, cross-sectional area (CSA), cortical thickness (CT) and cross-sectional moment of inertia (CSMI, an indicator of bone's bending strength) were derived from Hip Structural Analysis (HSA) from these scans. Participants with complete data were included, resulting in a sample of 1,536 (805 female).

In models adjusted for height, fat and lean mass, later walking age was associated with lower total hip BMC in males (-0.59% per month increase in age at walking, 95% CI: -1.00% to -0.18%, P = 0.005). This appeared to result from associations with BA (-0.35%, 95% CI: -0.60% to -0.10%, P = 0.007) but not BMD (P = 0.24). Later walking age was also associated with lower FN CSMI in males (-0.70%, 95% CI: -1.37% to -0.03%, P = 0.04) but associations with FN CSA were fully attenuated after adjustment for lean mass, and there were no associations with FN BMD or CT (all P > 0.15). In females, associations with walking age were only observed for FN CSMI (-0.66%, 95% CI: -1.25% to -0.07%, P = 0.03).

These results suggest that associations between early motor development and bone strength indicators may persist into older age, particularly in males. Greater hip BMC in early walkers appeared to be attributable to differences in BA not BMD. Whilst FN bending strength was also greater in early walkers, it was unclear whether this was due to advantages in bone width or cortical thickness. Interventions aimed at promoting development of early motor skills may be effective in promoting lifelong bone health.

P082

The emerging role of P2X7 receptor in mediating subchondral bone changes during mechanically induced osteoarthritis.

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Osteoarthritis (OA) can develop due to changes in joint mechanics that ultimately cause cartilage surface wear, chondrocyte apoptosis and subchondral bone (SCB) alterations. Little is known about the pathogenesis underlying development of OA and the role of SCB in that process. The P2X7 receptor (P2X7R) is an interesting target for OA as the severity of collagen-induced arthritis is reduced in P2X7R$^{-/-}$ animals. We hypothesized that excessive mechanical stimulation of cells drives P2X7R activation, leading to OA development and SCB remodeling. In this study we provide evidence that P2X7R$^{-/-}$ mice are less susceptible to mechanically induced changes in early OA.

The experimental design was based on a loading regime effective to induce OA in knee cartilage. Wild-type (WT) BalbC and P2X7R$^{-/-}$BalbC mice (males) aged 11 weeks were used. Axial compression was applied (Bose) to the limbs under general anaesthesia, thrice a week for 2 weeks (40 rounds; 9N peak load; 2N resting load). WT (n=6), P2X7R $^{-/-}$ mice (n=6) and unloaded controls (n=3) were sacrificed 3 days thereafter. Joints were scanned with microCT and tibiae SCB analysed.

P2X7R $^{-/-}$ tibiae displayed significantly higher SCB plate thickness in the medial compartment compared to WT controls (p=0.0087). Similarly, lateral and medial SCB trabecular parameters including bone volume (p=0.0152, p=0.0022, resp.), trabecular thickness (p=0.0022, p=0.0087, resp.) and trabecular lattices connectivity (p=0.0152, p=0.0043, resp.) were higher in P2X7R$^{-/-}$ animals. Loaded WT tibiae displayed enhanced osteophyte formation compared to P2X7R$^{-/-}$ tibiae. Unexpectedly, 2 weeks of loading caused a significant decrease in SCB plate thickness, in both lateral and medial compartments, of WT mice (p=0.0043 and p=0.0087, resp.). Bone remodelling was increased in the lateral SCB as demonstrated by a decrease in bone volume (p=0.0087) and trabecular number (p=0.0043), alongside an increase in trabecular separation (p=0.0043) and connectivity (p=0.026). No significant differences were present in P2X7R$^{-/-}$ tibiae.

In summary, we speculate that the loss of SCB architecture in loaded WT tibiae might reflect a transient stage that precedes SCB thickening. SCB is significantly altered in unchallenged P2X7R $^{-/-}$ mice and this might explain the reduction in bone remodelling in response to mechanical stimulation preventing development of OA.

P083

Syndecan-3 is important for maintaining bone mass

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Syndecan-3 (Sdc3) is a transmembrane heparin sulphate proteoglycan receptor, which is differentially expressed during skeletal development, with high expression in osteoblasts in the diaphyseal periosteum. However, little is known about the role of Sdc3 in the adult skeleton. We have observed that Sdc3-/- mice have increased bone fragility with ageing.
To test the hypothesis that Sdc3 regulates bone metabolism, we characterised the long bones of 6 month old Sdc3-/- (N=7) and WT (N=7) males by uCT. There was a significant decrease in bone volume (BV/TV, 43%, $p<0.01$), trabecular thickness (Tb.Th, 19%, $p<0.01$) and trabecular number (Tb.N, 28%, $p<0.01$) and an increase in trabecular pattern factor (Tb.Pf, 115%, $p<0.01$) in the Sdc3-/- compared to WT mice in keeping with an osteoporotic phenotype. In order to ascertain whether this premature bone loss was due to accelerated ageing of the skeleton we also assessed the long bones of 3 month old Sdc3-/- (N=5) and WT (N=7) males. There was a decrease in BV/TV, Tb.Th and Tb.N of 31% ($p<0.01$), 12% ($p<0.01$) and 21% ($p=0.02$) respectively, and increase in the Tb.Pf and the SMI of 50% ($p<0.01$) and 20% ($p<0.01$) respectively in Sdc3-/- as compared to WT mice. Cortical thickness and overall bone diameter were decreased by 10% ($p=0.01$) and 12% ($p<0.05$) respectively in Sdc3-/- as compared to WT mice. Although both the 3 month and 6 month old Sdc3-/- mice displayed a low bone mass phenotype, the reduction was greater in the 6 month old mice, suggesting a phenotype that worsens with age. In preliminary in vitro experiments we found an approximately 50% decrease in the number of osteoclasts generated from Sdc3-/- compared to WT ($p<0.01$), contrary to expectations. This suggests that the low bone phenotype in the Sdc3-/- mice is due to reduced bone formation rather than increased bone resorption.
In summary we have shown that Sdc3 plays an important role in maintaining bone mass. Lack of Sdc3 leads to a low bone mass phenotype in young adult mice, and accelerated bone loss with ageing. The low bone mass is likely due to impaired differentiation and/or function of osteoblasts.

P084

Microstructural analysis of disuse-related osteoporosis in an animal model of spinal cord injury

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Objectives: This study describes the micro-architectural changes that occur in the limbs of a rodent model of disuse osteoporosis resulting from a spinal cord injury (SCI). Studying this model will improve our understanding of disuse-related bone loss in patients with complete SCI, which arises from the removal of muscle-driven dynamic stimulation of bone.
Methods: Sixteen young male Wistar rats (Harlan, UK) (body mass 200-250g) were assigned randomly in to two groups. One group was given a transection of the spinal cord at thoracic level T9 (n=8), while the other group were sham-operated (n=8). 16-weeks post injury all rodents were sacrificed. Subsequently micro-Computed Tomography (microCT) scans of the distal femurs were taken at 70 KVp and 6.89 µm voxel size. Scaled volumes of interest in the distal metaphyseal trabecular and mid-diaphyseal bone were selected. Trabecular and cortical bone morphometric parameters were quantified and compared. The trabecular morphometric parameters analysed were trabecular bone volume fraction; mean trabecular thickness, separation and number; structural model index; trabecular pattern factor; degree of anisotropy and trabecular extent. The cortical morphometric parameters analysed were total cross-sectional area inside the periosteal envelope, cortical bone and medullary canal areas and mean cortical thickness.
Results: Compared to the control group, SCI appeared to lead to: i) 42% lower trabecular volume ($p<0.001$), characterised by 44% reduction in trabecular number ($p<0.001$), ii) 2% longer bones ($p = 0.037$) and iii) 15% smaller bone cross-sectional areas ($p<0.001$).
Conclusion: This study indicates that T9 transection leads to longer, thinner and potentially weaker distal femurs, and provides a useful model for disuse osteoporosis in SCI.

P085

The Fibrodysplasia Ossificans Progressiva (FOP) Connection Registry: patient reports of new bone growth

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Objectives

The objectives of the Registry are to organize the international FOP community for participation in clinical trials; to enable FOP patients worldwide to report data in a shared forum; to improve the collective understanding of FOP natural history; and to advance the understanding of FOP treatment outcomes.

Methods

The Registry is a global, non-interventional, voluntary database that captures demographic and disease data directly from FOP patients and their caregivers via a secure, web-based patient portal.

Results

As of 24 February 2016, 95 individuals with FOP submitted baseline information about their FOP disease at the time of their enrollment into the Registry. The average age of these participants is 24.6 years (age range = 1-71 years). Eighty-nine percent (85/95) of participants provided information about their life-long medical history of new bone growth. Among these 85 participants, 98% (83/85) reported greater than one body location or joint has formed new bone growth.

Each of the 85 participants was able to report information about new bone growth at 27 body locations and joints, providing data on 2,295 (85x27) individual body areas. Among the 2,295 individual body locations and joints, 45% (1,026/2,295) have been affected by new bone growth. The neck, upper back, lower back, and both shoulder joints were the most often reported locations affected by new bone growth. Among the 1,026 affected body locations and joints, 87% (893/1,026) have a partial or total loss of mobility. Future analyses will include evaluating any correlations between participants' ages and new bone growth and between episodic flare-ups and new bone growth.

Conclusion

Participant reports of new bone growth allow us to better quantify the severe impact that new bone growth has on an individual's quality of life and his or her ability to perform the routine activities of daily living. In addition to the information on new bone growth and its impact on mobility, the growing pool of data confirms the global FOP community's commitment to sharing their individual stories with the Registry in support of its FOP research objectives.

P086

Time trends in fracture incidence in the United Kingdom between 1990 and 2012: a CPRD Study

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Introduction

Evidence is accumulating of global secular changes in age- and sex-adjusted fracture incidence. In developing populations, such observations suggest increasing rates of fracture, however in developed countries fracture rates appear to be stabilising or even decreasing. Since altered fracture rates have major implications for healthcare provision, planning and resource allocation, we investigated secular changes to age- and sex-adjusted fracture risk amongst the UK population aged 50 years or above from 1990 to 2012.

Methods

To investigate changes in fracture incidence, we undertook a retrospective observational study using the Clinical Practice Research Datalink (CPRD), which contains the health records of 6.9% of the UK population. Fracture type was categorised according to the ICD-9 classification, and site-specific fracture incidence was calculated by calendar year for men and women. Linear regression analysis was used to calculate mean annualised change in absolute incidence. Mean rates in the first and last 5 years of the period were calculated and compared.

Results

Overall, fracture incidence was unchanged in both women and men from 1990 to 2012. Though the incidence of hip fracture remained stable amongst women (1990-1994: 33.8 per 10,000 py; 2008-2012: 33.5 per 10,000 py; p trend annualised change in incidence=0.80), it rose in men across the same period (10.8-13.4 per 10,000py; p=0.002). Conversely, clinical vertebral fractures became more common in women (8.9-11.8 per 10,000py; p=0.005) but remained static in men (4.6-5.9 per 10,000 py; p=0.72). The frequency of radius/ulna fractures did not change in men (9.6-9.6 per 10,000py; p=0.25), but, in contrast, became less frequent in women (50.4-41.2 per 10,000py; p=0.001). Secular trends amongst fractures of the carpus, scapula, humerus, foot, pelvis, skull, clavicle, ankle, patella and ribs varied according to sex and fracture site.

Conclusion

Although overall sex-specific fracture incidence in the UK population aged 50 years and over appears to have remained stable over the last two decades, there have been noticeable changes in rates of individual fracture types. Given that the impact of a fracture on morbidity, mortality and health economy varies according to fracture site, these data inform the provision of healthcare services in the UK and elsewhere.

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