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Poster Abstracts

Cancer

Targeting hepatocyte growth factor using Tivantinib combined with chemotherapy on myeloma *in vitro*.

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Abstract

Rationale and hypothesis: Multiple myeloma (MM) is a haematological malignancy arising from differentiated B-lymphocytes known as plasma cells. Myeloma cells create an autocrine loop by activating hepatocyte growth factor and its receptor c-met, stimulating their own growth. Tivantinib is a small molecule inhibitor of c-met and has been previously shown to have anti myeloma effects *in vitro* and *in vivo*. We hypothesised combining Tivantinib with chemotherapy agents already used in the treatment of myeloma will lead to synergistic anti-myeloma effects.

Methodology: Myeloma cell lines (JJN3,U266 and OPM2) were incubated for 48 hours before treatment with Bortezomib (10nm, 5nm, 2.5nm, 1.25nm, 0.625nm, 0.3125nm), Dexamethasone(1mm, 100µm, 10µm, 1µm, 100nm, 10nm, 1nm), Melphelan(1mm, 100µm, 10µm, 1µm, 100nm, 10nm, 1nm), or Lenalidomide(1mm, 100µm, 75µm, 50µm, 25µm, 10µm, 1µm). Cell viability was measured after 72 hours incubation by Alamar blue assay. The half maximal inhibitory concentration doses of Bortezomib and Dexamethasone were combined with Tivantinib(1µm) to investigate for synergistic effects.

Results: The half maximal inhibitory concentrations were 5nm Bortezomib, 45µm Dexamethasone, 20nm Melphalan. Lenalidomide had a poor *in vitro* response. Combination of Tivantinib (1µm) with half maximal inhibitory doses of Bortezomib (5nm) and Dexamethasone (45µm) resulted in a synergistic reduction of MM cell viability. Cell viability was reduced to 10% compared with control, with a combination index of 0.5 (strong synergism). RNA analysis investigating the mechanism identified apoptosis as a key mechanism.

Conclusions: Bortezomib and Dexamethasone have a synergistic anti-myeloma effect when combined with Tivantinib. Future work will investigate these anti-myeloma effects using an *in vivo* model of myeloma. The eventual aim would be to translate the use of Tivantinib as a combination therapy in clinical trials.

Osteosarcoma cells modulate skeletal stromal cell phenotype- site specific interactions

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Abstract

The tumour microenvironment plays a key role in the growth and progression of all cancers, including Osteosarcoma (OS); however, the cellular interaction of bone structural and immune cells with OS tumours is still poorly understood. OS occurs most often at the metaphyses of long bones, which has a different cellular composition to other compartments of the bone including the bone epiphysis and diaphysis.

In order to determine how skeletal location influences cellular composition, human bone marrow stromal cells (BMSCs) were harvested from the femoral diaphysis (FD), and femoral epiphysis (FE), of patients undergoing elective hip replacement surgery, and analysed for osteogenic differentiation. The cell types were subsequently co-cultured with OS Saos-2 cells in 0.4µm transwell plates to evaluate phenotypic and functional differences resulting from OS signalling. FD and FE cells were compared from at least three patients.

A 7 day assay comparing FD and FE cells in basal and osteogenic media revealed a lower expression of specific alkaline phosphatase (ALP) activity in the FE cells, indicating reduced osteogenic differentiation. FD basal 17.18 +/- 1.91, FE basal 5.13 +/-0.20, FD osteogenic 15.03 +/- 0.24, FE osteogenic 6.26 +/- 1.6, mean +/- SD, all units Avg Spec Activity nmols pNNPP/ng DNA. This pattern was also observed following a 28 day mineralisation assay, where FE cells expressed significantly fewer calcified bone nodules, supporting the ALP assay data. Co-culturing BMSCs with Saos-2 cells also resulted in a reduction of ALP in both FD and FE cell types, indicating an inhibition of osteogenic differentiation. Further evidenced by a decrease in gene expression of the osteoblast markers ALP and Osterix.

Together, these data provide initial insights into the effect of OS cells on BMSCs isolated from differing regions of the bone; factors that will be important in developing effective future therapies for OS.

Extracellular pyrophosphate: more than just an inhibitor of bone mineralisation?

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Abstract

Extracellular pyrophosphate (ePP_i) is well known for its role as a physiochemical inhibitor of bone mineralisation. However, information describing the direct actions of ePP_i on bone cells is limited. This study used mouse osteoblasts and osteoclasts to investigate the effects of ePP_i (1-100mM) on cell function, survival and gene expression. ePP_i (100mM) decreased osteoclast formation and resorptive activity by ≤50% (p<0.001). These inhibitory actions were associated with reduced mRNA expression (p<0.05) of genes involved in osteoclast fusion (DC-stamp) and bone resorption (Cathepsin K, TRAP, carbonic anhydrase II). Bone mineralisation was decreased 45% by 1mM ePP_i and completely abolished at ≥10mM, with no effects on osteoblast viability. Concomitantly, ePP_i increased the mRNA expression of osteoblast (Runx2, osterix, osteocalcin) and early osteocyte (E11, DMP1) marker genes and mineralisation inhibitors (osteopontin, matrix gla protein) (≤3-fold, p<0.05). ePP_i levels in bone are regulated by the coordinated actions of tissue non-specific alkaline phosphatase (TNSALP) and ecto-nucleotide pyrophosphatase/phosphodiesterase 1 (NPP1). In osteoblasts and osteoclasts, ePP_i reduced NPP1 expression (p<0.01). It also increased osteoblast TNSALP expression (≤2.5-fold, p<0.05) and activity (≤35%, p<0.05). Interestingly, we also observed that osteoclasts express TNSALP and exhibit low levels of enzyme activity although these were unaffected by ePP_i. The breakdown of extracellular ATP by NPP1 represents a key source of ePP_i: constitutive ATP release from bone cells was decreased ≤60% by ePP_i (p<0.001). This inhibitory effect was lost in the presence of pertussis toxin (an inhibitor of GPCR signalling). Finally, ePP_i causes a rapid decrease in intracellular cAMP levels (≤35%, p<0.001). Taken together these findings show that, in addition to its established actions, ePP_i exerts cell-specific effects on gene expression and function. The ability of ePP_i to alter constitutive ATP release and the expression/activity of enzymes involved in its metabolism also suggests that cells can detect ePP_i levels and respond accordingly.

Clinical

The impact of mild-moderate chronic hyponatremia on falls and fractures among osteoporosis patients

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Abstract

Background: Hyponatraemia is the commonest electrolyte imbalance among older people and has been associated with higher frequency of falls and fractures in acute setting. When mild to moderate and not associated with haemodynamic changes its effects are often unseen and sometimes called asymptomatic

Objective: to determine the prevalence of mild to moderate hyponatraemia in community dwelling osteoporosis patients and study its potential effects on reported falls and .

Design: 5 year retrospective study of community-dwelling osteoporosis patients in the North East of England to identify prevalence of mild-moderate "asymptomatic" hyponatraemia and study outcomes of falls and fractures among those patients.

Results: more than 51000 primary care records were screened. 706 patients had documented osteoporosis and were free living in community. 61 patients had mild-moderate haemodynamically asymptomatic chronic hyponatraemia. Those patients had three fold increase incidence of falls and 2.5 times incidence of new fragility fractures than non-hyponatraemia patients independent of other predisposing factors and co-morbidities.

Conclusion: Chronic hyponatraemia is associated with increased risk of falls and fractures among osteoporosis patients. The term asymptomatic chronic mild hyponatraemia is probably misleading and more research about sodium effects and osteoporosis are need.

Under-reporting of Osteoporotic Vertebral Fractures on CT Imaging in a Tertiary Oncology Hospital

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Abstract

Objective: Oncology patients are at risk of osteoporotic and metastatic vertebral fracture (VF). Osteoporotic VF predicts future fracture and mortality. Up to 70% of osteoporotic VF are not reported on imaging reports according to national data¹ but there are minimal data in an oncology population.

Method: A baseline audit was performed in a tertiary oncology hospital in accordance with ROS guidance¹. 100 CT thorax, abdomen, pelvis (CT-TAP) scans (patient age≥50yrs) were randomly selected (01/05/2019 to 31/05/2019 (n = 1914)). Sagittal reconstruction images were reviewed for moderate/severe VF under supervision of a consultant radiologist and compared to original report. Report parameters evaluated were: (a)comment on thoracic/lumbar spine appearance, (b)correct identification of moderate/severe VFs, (c)correct terminology, and (d)appropriate recommendations for further assessment¹

Results: 16/100 scans with osteoporotic VFs were identified (metastatic VF excluded). 13/100 reports commented on thoracolumbar spine appearance, 8/16 reported moderate/severe VF's correctly; correct terminology noted in 5/8 of correctly reported VF. No scans had recommendations for further assessment. Potential reasons for non-reporting were assessed; 2/8 patients died soon post-scan from progression of malignancy. The remaining 6/8 had stable disease.

Conclusion:

The incidence of osteoporotic VF (16%) on CT scan in this oncology population reflects reports in the general population². In our study only 50% of these were reported correctly and no reports suggested further assessment. Secondary prevention of vertebral fractures is efficacious and cost-effective and could be implemented in oncology patients with stable disease. These data will be used to inform a Quality Improvement initiative to improve management of VF for this patient group.

¹ *Clinical Guidance for the Effective Identification of Vertebral Fractures, ROS 2017.*

² *Bartalena T et al, Incidental vertebral compression fractures in imaging studies: Lessons not learned by radiologists World J Radiol 2010 28:399-404*

Bone pain in multiple myeloma - a translational study

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Abstract

Background: Pain remains one of the most feared and debilitating symptoms of multiple myeloma (MM) patients, and cancer located in the bone is especially painful and challenging to treat with conventional analgesics. Despite this, research into the nociceptive mechanisms of bone cancer pain in MM is sparse.

Aims: To provide a deep characterization of the self-reported experience of bone pain and quality of life of firstly-diagnosed MM patients, and after first-line treatment. To evaluate the effect of myeloma cells on the presence, location and density of sensory neurons in bone and its correlation to pain, at baseline and following first-line treatment. To correlate changes in systemic biomarkers of bone turnover and inflammation to pain, at baseline and following first-line treatment.

Methods: Patients undergoing a diagnostic trephine bone biopsy for suspected MM at Sheffield Teaching Hospital (Sheffield, UK) between October 2019 and October 2020 are invited to participate. Consenting patients are provided with 7 standardize questionnaires assessing pain, quality of life and catastrophizing. Serum samples of fasting patients are collected, and the research team is granted access to bone biopsies upon completion of medical evaluation. Patients with a positive MM diagnosis are invited to a follow-up upon completion of first line treatment, where questionnaires, blood samples and bone biopsies are collected again. The levels of bone turnover and inflammatory biomarkers in serum will be analyzed through enzyme-linked immunosorbent assay and multiplex cytokine arrays, respectively. Bone innervation will be assessed by immunohistochemistry.

Results: To date (02/2020), 11 patients have been recruited.

Discussion: This will be the first deep characterization of bone pain in MM patients and its correlation with disturbances in bone innervation. Understanding how bone turnover and inflammation correlate to bone pain in MM is crucial to identify novel analgesic targets for this condition.

Ethics: REC: 19/YH/0319.

High risk of hip fracture and hip fractures saved - a post hoc analysis of the SCOOP study

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Abstract

The SCOOP study compared usual care to a FRAX-based screening strategy, whereby anti-osteoporosis treatments were targeted to women age 70-85 years at high risk of hip fracture. In the screening arm, 14.6% of the women were allocated to the high risk group. Over the course of 5 years, screening prevented 54 hip fractures compared with usual care ($p=0.002$). The present analysis examined the pattern of prevented hip fractures using observed (O) and expected (E) hip fracture rates.

Five-year probabilities of hip fracture were calculated, without the inclusion of femoral neck BMD, using an adaptation of the FRAX UK model. In the 6250 women in the usual care arm, a total of 212 women with incident hip fractures were expected, with a total of 218 fractures actually observed (O/E 1.03, 95% 0.90-1.18). In the 6233 women in the screening arm, 212 women with incident hip fractures were also expected, but only 164 were observed (O/E 0.77, 95% 0.66-0.90), a reduction of 48 hip fractures. Within the screening arm alone, 142 hip fractures were expected in the 5335 women (rate 2.7%) deemed not to be at high risk of hip fracture, with 125 hip fractures observed (O/E 0.88, 95% 0.73-1.05). In contrast, in the 898 women categorised at high risk and recommended for treatment, 70 hip fractures were expected (rate 7.8%) but only 39 were observed ((O/E 0.56, 95% 0.40-0.77).

Screening by FRAX hip fracture probability is associated with a significant reduction in hip fractures. The trend for a small non-significant reduction in those not deemed at high risk may infer an independent effect of screening on hip fracture risk, though this requires further exploration. However, the majority of hip fractures prevented in SCOOP arose from the women designated to be high risk and recommended for anti-osteoporosis treatment. These results support the use of FRAX as a gateway for screening in women age 70 years or more.

Developing a Patient Questionnaire to Improve Osteoporosis Risk Factor Assessment in a Metabolic Bone Clinic

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Abstract

Background: Osteoporosis is a major global health problem. The thorough assessment of patients' risk factors for osteoporosis is crucial to aid clinical management decisions, but can be time consuming in a busy outpatient clinic.

Aim: To improve osteoporosis risk factor assessment and time-efficiency in the metabolic bone clinic at St. Mary's Hospital, London.

Methods: Clinic letters of new patients attending the metabolic bone clinic from August to October 2019 were screened for documented assessment of the following risk factors: smoking history, alcohol history, exercise, family history of hip fracture, calcium intake from diet, age at menarche and age at menopause. A patient questionnaire for osteoporosis risk factors was subsequently developed. This was completed by new patients attending from January 2020. Their resulting clinic letters were screened for the above risk factors. The time efficiency of the intervention was assessed subjectively using a questionnaire.

Results: The introduction of the patient questionnaire in the metabolic bone clinic led to an increase in documentation of the following risk factors for osteoporosis: family history of hip fracture (from 41% to 62.5%), calcium intake from the diet (from 52% to 75%), age at menarche (from 62% to 100%) and age at menopause (from 72% to 100%). The documentation of 'alcohol history' was similar (from 74% to 75%) and the documentation of 'smoking history' and 'exercise' was lower (from 85% to 75% and from 59% to 50% respectively) after the introduction of the patient questionnaire. 75% of clinicians (3 out of 4) reported that the patient questionnaire improved time efficiency.

Conclusions: Introduction of a patient questionnaire in a busy outpatient clinic can improve the completion rate of thorough osteoporosis risk factor assessment for osteoporosis and make the clinic more time efficient. Work is ongoing to further improve risk factor collection by optimising the questionnaire.

Engineering

The role of subchondral circulation in the physiology of osteoarthritis

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Abstract

Intraosseous pressure (IOP) has been studied for decades. It is usually raised in arthritis and bone pain but was of little clinical value. There are no accounts of IOP being measured during loading.

IOP was studied in an animal model. At rest IOP was found to be variable and sensitive to measurement technique. It had underlying cardiac, respiratory and circulation waves. It was correlated with blood pressure and had a proportionate pulse pressure (PP). Subchondral IOP was raised by proximal venous occlusion and loading and reduced by proximal arterial occlusion. Steroid treatment increased IOP but probably by reducing intraosseous fat volume.

Previously undescribed vascular marks on MRI scans were found in the axial subchondral plane and were found to be diminished or lost in early OA, $p < 0.002$.

An in vitro calf foot model was developed and used to further investigate IOP at rest and with combinations of perfusion. The model was then used to study different static and walking or dynamic loading patterns. Load was found to be transferred partly by hydraulic forces from subchondral to cortical bone through soft but high pressure tolerant fatty subchondral tissue. Histology confirmed the presence of the axial plane MRI vessels and demonstrated possible juxta-cortical valves which aid high pressure transmission, improving perfusion with repeated loading.

Axial subchondral histology in animal and human tissue was examined. Axial plane radiating vessels matching those seen on MRI were found. The vessels were present in healthy bone but absent in osteoarthritis. A subcortical valve like structure was identified which may allow high pressure hydraulic force transmission through delicate subchondral fatty tissues without damage.

Failure of vasculo mechanical circulation in the subchondral region may contribute to the development of osteoarthritis. This understanding offers hope for future osteoarthritis research and treatments.

Epidemiology

Imminent fracture incidence among index fracture patients with multiple myeloma relative to general population controls: a parallel cohort study of the UK CPRD

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Abstract

Objectives: To describe the imminent fracture rate within one and two years after an index fracture among multiple myeloma (MM) patients compared to patients without MM

Materials and methods: The Clinical Practice Research Datalink (CPRD) GOLD was used to identify a cohort of MM patients (1995-2017) in primary care, along with a cohort of non-MM patients, matched (1:4 ratio) on age, gender and GP practice at date of MM diagnosis (index date). Exclusion criteria applied to initial cohorts were: prior fracture as assessed in year before cohort entry, prior Paget's disease, prior cancer (excluding MM), less than 3-year look-back period prior to index date. These cohorts were then sampled for patients diagnosed with an index non-open fracture (excluding skull, face and digits) from between two years prior to index date and up to two years after. Patients were followed from date of first fracture to the earliest of either subsequent fracture at a major site (hip, spine, forearm & humerus), death, transference out of practice or two years after first fracture. Cumulative incidence at one and two years from index fracture were estimated accounting for the competing risk of death, along with associated sub-hazard ratios adjusted for age and gender.

Results: This analysis of index fracture patients included 413 patients from the MM cohort (at average age of 72 [SD: 11.5] years and of whom 58% were female) and 399 from the non-MM cohort (at average age of 75 [SD: 10.2] years and of whom 71% were female). The mortality at 2 years from index fracture was 21% and 7% in MM and non-MM, respectively. Results are included in table (below).

Imminent major fracture risk among index fracture patients: stratified by multiple myeloma (MM) status								
Imminent fracture outcome	MM cohort (n=413)		Non-MM cohort (n=399)		Age & sex adjusted sub hazard ratio			
	Number	Cumulative Incidence: % (95%CI)	Number	Cumulative Incidence: % (95%CI)	estimate	Lower 90% CI	Upper 95% CI	P-value
within 1 year of index fx	17	4.2 (2.5 to 6.4)	11	2.8 (1.5 to 4.9)	1.60	0.78	3.32	0.20
within 2 years of index fx	33	8.3 (5.9 to 11.3)	19	5.1 (3.2 to 7.7)	1.73	1.00	3.00	0.052

Conclusions:

In addition to increased mortality, MM may confer a significantly increased risk of imminent subsequent fracture following an index fracture. Further work is needed to confirm these findings

Miscellaneous

Increased arginine metabolism is a key signature of osteoblastic proliferation and differentiation

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Abstract

Knowledge of the metabolic processes that regulate osteoblastogenesis is important in understanding bone formation in health and its dysregulation in disease. Non-targeted metabolomics enables profiling of hundreds-to-thousands of metabolites simultaneously, and is therefore an attractive approach to study the global metabolic profiles of bone cells at a particular point in time or under given experimental conditions.

The metabolomic profiles of osteoblast-like cell lines were compared in order to gain insight into the metabolic changes that accompany the process of differentiation. Media samples were analysed by non-targeted LC-QTOF-MS (mass range 50-1700) and metabolite profiles were compared between MG63, TE85 and SaoS2 osteoblast-like cell lines, which represent the sequential order of osteoblast differentiation, in addition to C20 chondrocyte cell line and primary human osteoblasts.

The global metabolic profiles of each cell type were unique, as the samples from each group clustered separately in principal components analysis. The most striking difference was between TE85 and the other cell types. A total of 18 metabolites had significantly altered abundance (false-discovery rate-adjusted $P < 0.05$) in TE85 compared with all other cell types; 15 increased, 3 decreased. Pathway analysis indicated that a significant proportion of these metabolites mapped onto arginine metabolism, indicating a network of alteration consistent with increased arginase and nitric oxide synthase (NOS) activity. Arginine was decreased (min \log_2 fold change [LFC] = 10) in TE85 with concomitant increases in ornithine (min LFC = 1.65) and glutamate (min LFC = 0.3), metabolites derived from arginine via arginase, and increased citrulline (min LFC = 3.49), derived from arginine via NOS.

Together, these data indicate that the increased proliferative activity observed for TE85 osteoblast-like cells is supported by marked alteration to arginine metabolism via arginase and NOS activity. In addition, osteoblast arginase activity could also regulate arginine in the bone microenvironment which has recently been shown to control osteoclast formation.

Depth-related changes to bone mineralisation

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Abstract

The prevalence of osteoarthritis (OA) is rapidly increasing as a result of the aging population and factors such as obesity. The United Nations estimate that by 2050, 130 million people will suffer from OA and 40 million of these will be severely disabled [1]. It is currently unknown which of the joint tissues initiate the progression of OA. Primarily considered a disease of cartilage, research is now beginning to suggest that OA may begin in the bone. Depth-related changes in osteoarthritic bone have been studied by few papers, showing how subchondral and deep trabecular bone differ both structurally and biochemically. However, these changes have not been observed in healthy, aging bone.

This study examined the microarchitecture of femoral head cores in 10 x 2mm slices, via micro-CT (Figure 1). Specimens were from 35 donors (17 females, 18 males) aged 20-93 years old. Results confirmed previous work, which found that structural parameters were dependent on age and sex. Depth related changes were found in vTMD, where vTMD was at its lowest at 0-5% depth, peaking at 41-50% ($p < 0.0001$). No other depth-related changes were found in the healthy samples. At a depth of 0-6mm vs 10-16mm, no significant ($p > 0.05$) differences were seen in BV/TV, BS/BV, Tb.Th, Tb.Sp, vTMD and vBMD. In comparison, data from previous work on osteoarthritic femoral heads found significant differences in all comparative structural parameters at depths of 0-5mm vs 10-15mm [2].

Structural differences are believed to be caused by discrepancies in the biochemical and mechanical environment in which subchondral bone and deep trabecular bone are found. Structural differences in normal bone are not detectable via micro-CT but are in osteoarthritic bone. In contrast, changes to bone mineralisation are detectable with depth. These results suggest that most depth-related changes seen in osteoarthritic bone are a result of disease and not aging, whereas depth-related changes to mineralisation may be exacerbated in OA.

References

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Development of a novel deuterium oxide (D₂O) stable isotope tracer method for the quantification of bone collagen synthesis *in vivo*.

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Abstract

Developing safe and practical strategies to prevent weakening of bone tissue is vital, yet attempts to achieve this have been hindered by a lack of understanding of the short-term physiology of human bone collagen turnover – a process that directly underpins bone strength and quality. Little is known about how bone collagen responds to potentially favorable interventions (*e.g.*, exercise/diet) to improve bone strength, due to the lack of robust analytical approaches. To address this, we developed a method to quantify bone collagen turnover *in vivo*, using a deuterium oxide (D₂O) direct tracer incorporation technique. Male and female rats from a selectively bred rat model, exhibiting either low (LRT) or high (HRT) responses to endurance running training (enabling us to determine any effects of exercise capacity on bone collagen turnover) ingested D₂O for 3-weeks, in drinking water. Femur diaphyses were obtained at necropsy; after demineralisation, collagen proteins were isolated and hydrolysed in 0.1M HCl in Dowex H⁺ resin overnight at 110°C, and free amino acids eluted from the resin with 2M NH₄OH. Following this, amino acids were derivatised as their *n*-methoxycarbonyl methyl esters. Fractional synthetic rate (FSR) of collagen proteins was determined by incorporation of D₂O into protein-bound alanine via GC-pyrolysis-IRMS and body water enrichment by TCEA. Body water enrichment was stable over 3-weeks. Femur collagen synthesis rates (CSR) ranged 0.02-0.35 (0.13±0.08) FSR%/day⁻¹, being >20 fold lower than muscle protein synthesis rates measured previously in this cohort, and were greater in HRT compared to LRT rats (P<0.001, Figure 1). Therefore, direct measurements of bone collagen synthesis can be made in rodents over a 3-week timeframe using our D₂O tracer method. This method will be crucial to determining the responses of bone collagen to interventions, such as exercise, diet, sex, age and disease in rodent models, and has applicability to human investigations.

Muscle & Bone

Electrically-stimulated synergistic Recruitment of Antagonistic Muscle Pairs (RAMP-ES), to maximise bone stimulation in the paralysed limbs of people with spinal cord injury

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Abstract

Individuals with complete spinal cord injury (SCI) experience severe bone loss that leads to fragility fractures. The use of electrical stimulation to train paralysed muscles and recover bone loss has been limited by the low muscle forces elicited from atrophied muscles. Simultaneous stimulation of opposing muscle groups such as knee extensors and flexors could allow larger muscle forces to be applied to bone without resulting in undesirable large joint torques.

Therefore, we recruited 7 uninjured participants (2 males, 5 females, mean age 32.1 ± 5.7 y) to investigate the feasibility of this approach and measure the resultant knee joint net torque. One pair of surface electrodes was placed on each muscle group to deliver the electrical current (biphasic square pulses, 20-45 mA, 70 Hz, 300 μ s) from a handheld stimulator. The current was delivered first to the quadriceps and the hamstrings separately (single stimulation), before being applied to both muscles simultaneously (dual stimulation). Differences between torques in the three stimulation types (knee extensors, knee flexors and dual stimulation) were assessed with paired t-tests.

During single muscle stimulation, torques elicited by knee extensor stimulation were 100-310% greater than those elicited from knee flexors at different stimulation intensities ($P < 0.05$). In contrast, knee extension torque during single and dual stimulation was similar ($P=0.91$).

Simultaneous stimulation of opposing muscle groups appears feasible, without resulting in increased knee torques. Further tests should be conducted to aim to balance elicited muscle forces in order to reduce the net joint torque.

ASPIRE™: Computer-aided Opportunistic Identification of Vertebral Fractures in Computed Tomography Images

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Abstract

We describe ASPIRE™, an osteoporosis case-finding service designed to increase reporting rates for vertebral fractures (VFs) incidentally visualised in CT images. Osteoporosis is a common skeletal disorder and is associated with considerable increases in morbidity and mortality. VFs are an early manifestation and indicate a significantly increased risk of future fractures. However, VFs are under-reported, particularly when visualised incidentally in CT [1].

ASPIRE™ is a commercial, out-sourced service that runs in parallel with hospital-based radiology reporting. It uses machine learning software [2] to identify vertebral bodies in CT images and classify VFs (Fig. 1). The software can localise vertebral outlines with sub-mm accuracy, enabling automation of standard vertebral body height measurements. Diagnoses are reviewed by in-house radiologists, and a VF-specific report is automatically generated and returned to the requesting hospital and the patient's local Fracture Liaison Service (FLS).

Three prospective feasibility studies of ASPIRE™ were conducted at NHS hospitals, covering 12 months of CT imaging for patients aged over 50 (9797 patients; 50.1% female). ASPIRE™ reported VFs in 20.6% of patients (50.3% female); 33.0% of these were also reported by radiologists at the requesting hospitals but only 5.2% had been referred to their local FLS. As a result, ASPIRE™ referred 1944 patients for further management. We conclude that ASPIRE™ can identify significantly more VFs than hospital-based reporting, but that its automation of FLS referral may be an equally important route to patient benefit.

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[2] Proc. MICCAI CSI 2016, LNCS 10182:51-63.

Local fabric density in L2 vertebral body bone by high contrast resolution x-ray microtomography.

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Abstract

Many x-ray microtomography (XMT) studies of human vertebral bone samples have been conducted, but none with high quality, high contrast resolution methodology which necessarily require long integration times and, therefore, stable samples. We studied 69 ~2mm thick, parasagittal sections of L2 vertebral bodies preserved by embedding in PMMA under the aegis of the European Union BIOMED I study 'Assessment of Bone Quality in Osteoporosis'. Prior studies using quantitative backscattered electron [qBSE-SEM] imaging had shown a wide spread of local mineralisation density values at one cubic micrometer [1 fl] resolution, but only in 2D section planes. To acquire 3D data, we used the MuCAT2 TDI XMT system at 30µm voxel size, 90kV, typically 72h per scan, each scan corrected for beam-hardening and calibrated with a multi-metal calibration carousel, Linear Attenuation Coefficient (LAC) accuracy better than 2%. Results are expressed in LAC (cm⁻¹). Analysis used ImageJ Fiji. To circumvent partial volume artefacts, we stripped, in 3D, one voxel from all free bone surfaces. We compared distributions for whole bone slabs and regions selected to contain only internal trabecular bone, avoiding cortices and end plates. Both showed extreme ranges, but whole slab values contained higher proportions of highest density voxels. These results agree with the qBSE-SEM data which showed the highest values in calcified cartilage in end plates and calcified ligamentous inclusions and Sharpey fibre bone rich regions in cortices, particularly the anterior cortex. These regions will therefore artefactually appear to be thicker in lower resolution and clinical CT imaging. 'Bone' in the vertebral body is not one tissue but has a variety of fabrics and fabric densities.

New quantitative method for increasing information content in polarised light imaging of bone tissue

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Abstract

In linearly polarised light (LPL), birefringent structures appear brightest if they lie both in the plane of the section and at 45°/135° to the axes of the crossed polarising filter elements, but dark if perpendicular to the section plane or parallel to either polarizer or analyser, preventing measurement of the whole scene at once because nothing can be resolved in the dark sectors of the 'Maltese cross'. This may be solved using circularly polarised light (CPL), when dip with respect to the section-plane may be quantified for plane parallel sections and we can use pseudocolour to produce dip maps. CPL, however, does not differentiate between in-plane orientations. We provide a new solution by combining numbers of PLM images to map orientations in 3D. We have automated the coupled rotations of polarising and analysing filters at, for example, 3°, 5°, 7.5°, 10° or 15° intervals through a range of 90° - with digital LPL images recorded at each orientation - and exploit digital processing. For in-plane orientation mapping display we use the colour circle sequence Red, Yellow, Green, Cyan, Blue, Magenta, where colour shows the orientation with 4 repeat cycles in 360°. Brightness is proportional to the cosine of the dip/strike angle with respect to section plane, being brightest in plane, and black when perpendicular to that plane, i.e., parallel to the optic axis. The dip value can be displayed in a pseudocoloured version of the sum of the separate monochrome LPL images using a Look-Up-Table with six 15° classes.

The new method is powerful, label free and best used with unstained sections, but most stains do not interfere too much. Thus it may be used for much archival material. It proves to be excellent for undecalcified, uncalcified and decalcified tissue sections.

Displacing Sedentary Behaviour with light intensity activity improves bone mineral density in older females

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Abstract

Background: Sedentary behaviour (SB) and light-intensity-physical-activity (LIPA), are associated with bone health in older adults (1,2). Therefore, we sought to build on previous association studies, through quantifying alterations in bone mineral density (BMD) in response to two longitudinal LIPA accumulation interventions. **Method:** Thirty-six older females (aged 73±5yrs) underwent an overnight rested Dual X-ray absorptiometry (DEXA) whole body scan. BMD was quantified at six sites (arms, ribs, thoracic spine, lumbar spine, pelvis, and legs), and bone health classifications determined through T-scores (normal, osteopenic, and osteoperotic). Participants were randomly allocated to: SB fragmentation, performing 2 minutes of upright LIPA, for every 30 minutes spent sitting (SBF), Light intensity physical activity (LIPA), performing a single 45 minute continuous bout of upright light activity in the morning, or Control groups (CON). Measures were taken at weeks 0 and 8. **Results:** There was no between group difference in T-Score at baseline attesting to the well-matched study sample (One-way ANOVA, $p > 0.05$). After accounting for factors previously (1,3) identified as co-variates in this age group (Android:Gynoid ratio, total body fat, and body mass index), Thoracic Spine BMD was found to significantly change over time ($p \leq 0.05$) in the pooled population. Despite Thoracic Spine exhibiting no group×time interaction ($p = 0.20$), marked improvements in BMD were observed in the experimental groups (SBF: 4.0±12%, LIPA: 3.6±8.8%), but not control (-2.5±10.0%). Leg BMD exhibited a significant group dependant change over time ($p = 0.04$) with percentage change in the SBF, LIPA, and CON groups 0.5±1.2%, 0.2±2.2%, and -0.5±0.7% respectively. **Conclusion:** Displacing SB with LIPA may significantly alter BMD in older female. Especially interesting and novel, is our observation that breaking sedentarism throughout the day is potentially more effective at improving indices of bone health than carrying out a relatively similar volume of light physical exertion within a condensed session.

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Development of a machine learning-based fully automated hip annotation system for DXA scans

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Abstract

Introduction

Hip shape is an important risk factor for hip osteoarthritis and can be derived from dual X-ray absorptiometry scans (DXAs), which exist in large population cohorts. Unfortunately, manual shape measures are time-consuming to derive, preventing wider use in research and clinical practice. We aimed to develop a system for annotating hips automatically on DXAs using machine learning (ML).

Methods

A comprehensive hip shape outline was designed using 86 anatomically guided points placed around the proximal femur, trochanters and superior acetabulum. This was manually applied by 4 trained annotators to 2000 randomly selected left hip DXAs (of which 25% were chosen with self-reported osteoarthritis to encompass pathological joints) from UK Biobank. This produced a 'gold standard' annotation of each hip. 70 DXAs were excluded due to artefact, leaving 1930 participants/DXAs (mean age=62.7, SD=7.5, 981/949 females/males). Automatic point placement was implemented by training ML models on half the data with model performance evaluated on the remaining unseen images (Two-Fold Cross-Validation). The system was evaluated using the Euclidean distance between the automatic points and the manual curves (pt-crv error). We applied the system to right hips (image flipped). We used statistical shape models to analyse hip shape variations between females and males, and between left and right hips.

Results

The automatic-manual pt-crv error was below 0.66mm in 95% of hips. Figure 1 shows the difference between the mean female shape and the mean male shape for manual and automated annotations. In 95% of subjects the pt-crv distance between right hips and: (1) manually-annotated left hips was below 1.79mm, (2) automatically-annotated left hips was below 1.70mm.

Conclusions

We produced a fully automated system that placed points accurately around the hip as shown in DXAs. The reliability of this system will enable study of the hip joint shape in large population cohorts.

Single Cell RNA Sequencing of Human Bone Marrow reveals new targets for enrichment of Skeletal Stem Cells

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Abstract

The novel regenerative capacity identified in bone, is attributed to the existence of highly specialised skeletal stem cells (SSCs) with the potential to differentiate along the osteogenic, adipogenic and chondrogenic lineages. However, the successful application of SSCs in reparative medicine is limited significantly by the lack of specific target markers for isolation of SSCs from human bone marrow. Single cell RNA sequencing platform, Drop-seq, is a high-throughput methodology that utilises microfluidic systems to encapsulate single cells in droplets for parallel sequencing of thousands of single cell transcriptomes and subsequent identification of rare cell types within heterogeneous bone marrow populations and cell type-specific molecular signatures for delivery into isolation protocols.

Methods: We have used a protocol to isolate potential SSC/progenitor populations from human bone marrow using oligonucleotide-coated gold nanoparticles that isolate cells from heterogeneous populations based on the expression of target mRNA. We have performed Drop-seq on the enriched SSC populations, profiling >15,000 cells, to dissect the cellular heterogeneity within these populations and identify new targets for nanoparticle-based sorting. We have subsequently, assessed the differentiation and proliferation potential of the novel SSC-enriched populations.

Results: Delivery of the enriched SSC populations into the DropSeq methodology revealed cellular heterogeneity in the bone marrow populations. Differential gene analysis between cell types presented new distinct targets for SSC-isolation, which, when implemented into the initial nanoparticle-based cell sorting, showed significantly enhanced enrichment of SSC, compared to previous targets and other cell-sorting technologies, including magnetic cell-sorting using Stro-1 antibody.

Discussion & Conclusions: Nanoparticle-based cell sorting, combined with DropSeq, delivers enriched human SSC populations for enhanced sequencing depth and reveals novel targets for SSC-isolation protocols. These molecular targets, when implemented in isolation protocols, allow enhanced SSC enrichment and offer an improved source of SSCs for research and ultimately therapeutic evaluation.

The acute response of sclerostin and bone turnover biomarkers to whole-body vibration with blood flow restriction: a randomised crossover study

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Abstract

Background: Blood flow restriction (BFR), also known as vascular occlusion, may augment the skeletal response to whole-body vibration (WBV) and benefit populations unable to perform high impact exercise. No studies have examined the response of serum sclerostin to BFR when applied to exercise. This pilot study aimed to investigate the acute response of sclerostin and other serum biomarkers of bone turnover to WBV combined with BFR.

Methods: Ten healthy males (mean \pm standard deviation; age: 27 ± 8 years) completed two experimental conditions separated by 7 days in a randomised, cross-over design: (i) whole-body vibration (WBV; ten 1-minute bouts of WBV in a semi-squat position with 30 s recovery) or whole-body vibration with blood flow restriction (WBV + BFR; WBV protocol with the addition of a pressure cuff around each thigh [ten cycles of 110 mmHg inflation with 30s deflation during recovery]). Fasting blood samples were obtained pre-exercise (PRE), immediately post-exercise (POST), 1-hour post-exercise (POST 1H), and 24 hours post-exercise (POST 24H). Serum samples were analysed for sclerostin, cross-linking telopeptides of type I collagen (CTX-1), and bone-specific alkaline phosphatase (B-ALP), and the B-ALP/CTX-1 ratio was calculated.

Results: No significant time \times condition interaction occurred for sclerostin, CTX-1, or the B-ALP/CTX-1 ratio ($P > 0.05$). There was a significant time \times condition interaction for B-ALP ($P = 0.003$); B-ALP values at POST 24H were significantly greater following WBV compared to WBV + BFR ($P = 0.007$).

Conclusion: BFR applied to a single session of WBV does not significantly affect serum sclerostin or bone turnover biomarkers such as CTX-1 and B-ALP. Further research is required test the osteogenic potential of BFR strategies.

A new automated method of segmenting trabecular bone: investigating subchondral trabecular changes as a predictor of osteoarthritis at the joint surface

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Abstract

Analysing changes in bone structure gives us insights into key questions about how bone responds to the environment. Such questions range from how bone adapts to load, to how pathology causes alterations in bone structure. In order to analyse bone morphology, these investigations often require the separation (segmentation) of trabecular and cortical bone in CT datasets. However, to our knowledge no standardized protocol for such segmentation exists. We therefore developed an automated approach in Avizo to separate cortical and trabecular bone. We created a “recipe” in Avizo that automates the separation of trabecular and cortical bone with a single click, once the element of interest has been isolated (segmentation of the epiphysis is the only manual part). This new method reduces the user bias inherent to manual segmentation, and increases efficiency of data processing. It can be applied to a wide range of research projects.

Our aim is to investigate whether three dimensional trabecular changes in the epiphysis of STRort mice are related to osteoarthritic damage at the knee joint. Previous 2D studies have found a link between changes in subchondral trabeculae and the progression of osteoarthritis. 3D analysis provides a richer dataset to examine trabecular structure parameters. We are investigating BV/TV (bone volume/total volume), degree of anisotropy, trabecular thickness, predominant direction, spacing, surface area, as well as connectivity and nodal inter-trabecular angles. We will compare parameters in early and late stage osteoarthritic STRoRT mice, as well as in healthy mice, to examine whether trabecular changes beneath the joint correspond to and can predict damage at the joint surface.

Skeletal defects in murine models of secondary dystroglycanopathy

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Abstract

Muscular dystrophy patients suffer from poor bone health culminating in an increased fracture risk which is often ascribed to lack of mechanical loading due to muscle weakness. Secondary dystroglycanopathies are a sub-set of the muscular dystrophies, characterised by the defective glycosylation of alpha-dystroglycan with mutations in glycosylating factor Fukutin-related protein (FKRP) being the most common cause of disease.

Using micro-CT and histological analysis we investigated two murine models of FKRP deficiency: FKRP^{MD} and FKRP^{KD}. Bone architecture was assessed in neonatal femora of FKRP^{KD} mice and tibiae of 6 and 12-week-old male FKRP^{MD} mice. Cartilage integrity was assessed in 12-week-old FKRP^{MD} knee joints using toluidine blue staining and alpha-dystroglycan distribution was assessed by immunohistochemistry as well as FKRP expression using an additional murine FKRP-GFP reporter model.

We found deficits in bone volume ($p < 0.05$), trabecular number ($p < 0.05$), trabecular pattern factor ($p < 0.01$) in neonatal FKRP^{KD} femora compared to controls. 6-week-old and 12-week-old FKRP^{MD} tibiae also revealed deficits in trabecular bone compared with controls with significantly reduced bone volume and tissue volume (both $p < 0.05$). Analysis of cortical bone in 6-week-old male FKRP^{MD} mice revealed shorter tibiae with less bone and significant differences in indices of strength and shape in many regions of the diaphysis (eccentricity, I_{min}, I_{max} and predicted resistance to torsion, all $p < 0.05$) with the same trends observed in the tibiae of 12-week-old mice. Cartilage integrity remained intact while immunohistochemistry showed alpha-dystroglycan and FKRP distribution in skeletal tissues.

These results show that deficient glycosylation of alpha-dystroglycan in FKRP mutants produces deficits in bone mass and architecture which are consistent with weaker bones. As these deficits exist at birth prior to the onset of muscle damage and persist into adulthood prior to loss of muscle force, our data suggest a direct role for alpha-dystroglycan in the development and homeostasis of bone.

Hypophosphatasia in Adults at a Specialist Centre in the UK: The spectrum of musculoskeletal disease

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Abstract

Background

Hypophosphatasia (HPP) is a condition arising due to mutations in the gene encoding the tissue-non-specific alkaline phosphatase (TNSALP) isoenzyme (*ALPL*). There are over 300 known mutations associated with HPP, with great heterogeneity in clinical presentation.

Diagnosis is based on clinical features, biochemical abnormalities (low serum alkaline phosphatase (ALP), raised serum pyridoxal-5'-phosphate and raised urinary phosphoethanolamine), and genetic testing. The dentition and musculoskeletal system are commonly affected. Low trauma and poorly healing metatarsal and atypical subtrochanteric femoral fractures may be seen. In milder cases, individuals may present with calcium pyrophosphate deposition disease (CPPD) or non-specific musculoskeletal features.

This retrospective observational study, performed at a specialist centre, aimed to characterise the spectrum of musculoskeletal manifestations in adults with HPP.

Methods

Patient records of adults with a confirmed HPP diagnosis seen in the Rheumatology Department at St George's Hospital, London, were consulted. Clinical features at presentation and those developed subsequently were recorded, focusing on musculoskeletal manifestations. Biochemical, genetic and radiographic data were also obtained.

Results

Fifteen individuals (two male, thirteen female) with a confirmed HPP diagnosis were identified. Average delay to diagnosis was 21 years. The commonest presenting feature for the whole cohort was joint pain or swelling (most commonly of the upper limbs), followed by dental caries. Fractures were the commonest presenting musculoskeletal feature for those developing HPP in childhood. Other common musculoskeletal diagnoses were osteoarthritis (eight cases), tendinopathy (five cases), psoriatic arthritis (four cases), CPPD (three cases) and bursitis (three cases). Eight individuals reported poor mobility or abnormal gait, and three were unable to work through ill health.

Conclusion

There is a long delay to diagnosis of HPP. A low ALP and features such as fractures and CPPD should prompt consideration of the diagnosis. Earlier recognition may avoid potential harm through inappropriate bisphosphonate or high-dose vitamin D therapy.

Could fluid in a sinusoid flow into the intertrabecular marrow only with a sinusoidal fluid pressure?

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Abstract

The red marrow of intertrabecular pores (PIT) contains various bone cells, adipocytes, and extracellular fluid. In intracancellous marrow space, the marrow arteriole divides into capillaries. The capillaries have sinusoids, which are drained by venules. The sinusoids are average of 25 μm wide and have a thin wall. Sinusoid walls which are highly porous (2.5 %) and lined by endothelial cells are flown by fluid and traversed by hematopoietic cells in both directions (blood to marrow and conversely). Importantly, the sinusoids role as sources and drains of fluid in the PIT. The sinusoids uniformly distribute in the PIT as a density of 90~100 sinusoids per $3 \times 10^6 \text{ mm}^3$. The fluid pressure (FP) of sinusoids is unknown. However, it is reported that the intramedullary FP in metaphyses of animal long bone ranges from 1.78 to 2.73 kPa. Since the inner part of metaphysis is composed of the cancellous bone, the intramedullary FP could represent the sinusoidal FP (PITFPS). It is reported that the water content fractions of the marrow and blood have the same value of 0.8. As a result, an osmotic pressure equilibrium would be formed in the PIT. Therefore, the sinusoidal FP should generate intramarrow flow through the extracellular gaps and matrix pores. Since the cells and adipocytes are deformable, applications of the PITFPS through very permeable sinusoidal walls on the boundary between the marrow and sinusoids would cause local deformation of the cells and adipocytes. This could result in changes in configuration of the extracellular gaps. As a result, the intramarrow flow could be changed. In this study, a sinusoid surrounded by the adipocytes with the extracellular matrix was modeled. Then, a PITFPS of 2.73 kPa was applied to induce local deformation of the adipocytes (set as the bulk modulus of 9.5 kPa) and extracellular matrix. In addition, the intramarrow flow was analyzed using a multiphysics (COMSOL, MA, USA). The initial volume ratio of extracellular gaps was set as 0.016. When the sinusoidal FP is applied to the marrow, a compacting local deformation of the marrow is instantly occurred. The compacting deformation of the marrow induce the intramarrow flow to be nearly zero. As a result, the intramarrow flow through the extracellular space could not be possible. Consequently, the mass transport would not be possible for the metabolisms and various geneses. Therefore, a substantial mechanism is required to help the intramarrow flow for augmenting mass transport.

How do joints resist cracking under pressure?

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Abstract

Events preceding osteoarthritis (OA) are poorly understood, and scoring systems have low predictive power. Only late-stage OA is detectable by clinical imaging; gross anatomical changes are better characterised than micro-anatomical contributions and aetiopathogenesis is ill-defined. We establish proof-of principle for nano-scale synchrotron imaging of whole mouse joints, with emphasis on quantitative analysis of calcified cartilage (CC) micro-anatomy, in a well-established mouse model of spontaneous OA. We hypothesise that CC anatomical variation is an unacknowledged micro-anatomical OA risk factor with a biomechanical role in disease aetiology.

Str/Ort mice develop OA predictably with age, whereas their parental CBA strain model healthy joint ageing. Our streamlined nanoscale imaging protocol quantifies anatomical variation between Str/Ort and CBA knee joints, with focus across the entire CC compartment. Male mice were culled at 20wks (OA onset), hindlimbs were removed and frozen at -20°C until use. High resolution (0.8125µm) synchrotron computed tomographic (sCT) scans were obtained at the I13-2 beam (Diamond Light Source, Harwell) and analysed using CTAn and SPSS.

Our analyses reveal hitherto unquantified structural characteristics of CC. Relatively enlarged (*mean increase 213mm³; p<0.001*), less spherical (*Fig. 1A*) mineralised CC lacunae were measured in Str/Ort versus CBA mouse knees prior to any articular cartilage demise. 3D evaluation additionally reveals clustering of larger lacunae in thicker CC in the OA-prone medial tibial compartment of the joint Str/Ort mice. Our data confirms for the first time that these strains differ in hypertrophic phenotype; while healthy CC chondrocytes (*Fig. 1B*) increase in sphericity with greater hypertrophy, the opposite is true in pre-OA CC (*p<0.001; Fig. 1C*). Our data support the hypothesis that anatomical variants of CC may biomechanically predispose to subchondral cracking and sclerosis seen in OA. Future work will explore whether these imaging biomarkers will provide predictive power lacking in clinical practice.

Identifying the independent role of body weight and leptin deficiency on peripheral bone remodelling in vivo

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Abstract

Chronic obesity results in an increase in plasma leptin levels and concomitant increases in bone loading due to increased body weight. The role of leptin and load on bone remodelling of peripheral long bones in response to diet induced obesity is unclear. In this study we controlled for body weight and leptin by utilising three groups of aged and sex matched C57 and leptin deficient ObOb mice. C57 chow (n=4), C57 high fat diet (HFD) (n=4) and ObOb weight-paired chow (n=4) mice were studied until 24 weeks of age. The impact of body weight on bone remodelling was investigated using C57 mice fed a normal or HFD for 20 weeks. The role of leptin on bone remodelling was investigated, independent of body weight in C57 and weight-paired ObOb mice fed normal chow for 20 weeks. Whole tibiae were fixed prior to μ CT scanning (resolution 4.3 μ m, 0.7° rotation step) and reconstructed using NRecon. Post-reconstruction, CTAn was used to calculate percentage trabecular bone volume (BV/TV), trabecular number, trabecular separation, bone mineral density (BMD) and cortical bone volume (C.BV).

Compared to C57 lean controls, a significant increase in body weight in HFD C57 (Fig.1 A) resulted in a significant elevation in tibia cortical bone volume and trabecular separation (Fig.1 B&G). Bone mineral density of cortical and trabecular regions of the proximal tibia were also significantly increased in response to increased body weight (Fig.1 C&E). However, independent of changes in body weight, leptin deficiency resulted in significant reductions in bone volume of cortical and trabecular proximal tibia (Fig.1 B&D), along with a significant reduction in trabecular number (Fig. 1F). Independent of body weight, leptin deficiency did not significantly alter bone mineral density of cortical or trabecular proximal tibia (Fig.1 C&E).

These findings suggest that an increase in body weight increases bone density, however leptin appears to play a greater role in the maintenance of bone volume in these studies. Future studies will investigate the density and volume of ObOb HFD fed males to determine if reductions in bone volume and increased density are maintained at higher body weights.

Categorizing ten sports according to bone and soft tissue profiles in adolescents

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Abstract

Purpose: Considering the different loading and training characteristics of the sports practiced during growth, it is important to specify and categorize the bone and soft tissue adaptations in adolescent athletes. This study aimed to categorize ten different loading sports and a non-sport group and identify the differences in bone density and soft tissues. **Methods:** The sample included 625 adolescents (10 to 17 years old) of ten sports (soccer, basketball, volleyball, track & field, judo, karate, kung-fu, gymnastics, baseball and swimming) and a non-sport group. Dual energy X-ray absorptiometry assessed areal bone mineral density (aBMD), bone mineral apparent density (BMAD) and soft tissues (lean soft tissue and fat mass). The results were adjusted for sex, years from age of peak height velocity (PHV), lean soft tissue, fat mass and weekly training volume. **Results:** The comparisons among groups showed that soccer had the highest whole body aBMD (mean \pm SEM: $1.082 \text{ g/cm}^2 \pm 0.007$) and lower limbs aBMD ($1.302 \text{ g/cm}^2 \pm 0.010$). Gymnastics presented the highest upper limbs ($0.868 \text{ g/cm}^2 \pm 0.012$) and whole body BMAD ($0.094 \text{ g/cm}^2 \pm 0.001$). Swimming presented the lowest aBMD values in all skeletal sites (except at the upper limbs) and whole body BMAD. The soft tissue comparisons showed that soccer had the highest lean soft tissue ($43.8 \text{ kg} \pm 0.7$). The lowest fat mass was found in gymnastics ($8.04 \text{ kg} \pm 1.0$). **Conclusion:** The present study investigated and categorised for the first time ten different sports according to bone density and soft tissue profiles. Soccer and gymnastics sport groups found to have the highest bone density in most body segments and both sports were among the groups with the lowest fat mass.

Investigating the effect of obesity and testosterone treatment upon bone remodelling in APOE^{-/-} mice *in vivo*.

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Abstract

Atherosclerosis-prone apolipoprotein E-deficient (APOE^{-/-}) mice subjected to orchietomy were used to assess the effect of obesity and testosterone replacement therapy on bone remodelling *in vivo*. APOE^{-/-} mice develop multiple phenotypes associated with metabolic disease including atherosclerosis, hyperlipidaemia and increased body weight, associated with high fat diet induced obesity. Testosterone therapy has been shown to improve the metabolic phenotype of obese males with hypogonadism, however the effect of testosterone replacement therapy on bone remodelling is currently poorly understood.

Male APOE^{-/-} mice were split in to 3 groups: sham surgery followed by placebo treatment (control: n=9) and orchietomy followed by either placebo (n=8) or testosterone treatment (n=10). Surgeries were performed at 7 weeks of age, whilst high fat diet and testosterone/placebo treatment regimes began at 8 weeks of age. Treatments were administered via intramuscular injection once every other week for 17 weeks, and mice were sacrificed at 25 weeks of age. Tibias were scanned *ex-vivo* using μ -CT (4.3 μ m resolution, 0.7° rotation step over 180°) before post-reconstruction analysis of trabecular bone volume/tissue volume (%), thickness (mm), separation (mm) and number (mm⁻¹) as well as cortical bone volume (mm³) and porosity with post-analysis histology.

Orchietomised mice treated with placebo demonstrated significantly reduced tibia trabecular bone volume, number and thickness compared to control mice despite no significant differences in body weight. Tibia trabecular bone parameters were rescued back to control levels in orchietomised mice treated with testosterone. Tibial total cortical porosity was significantly reduced in orchietomised mice treated with placebo compared to controls, but remained at control levels in orchietomised mice treated with testosterone. Cortical bone volume did not significantly differ between groups.

Results suggest that testosterone plays an important role in remodelling of tibia trabecular bone independent of body weight, whereas the effect on cortical bone are less pronounced. Future work will utilise histology and immunohistochemistry to determine the prevalence of osteoblasts, osteoclasts and marrow adipocytes in the proximal tibia of APOE^{-/-} mice. Furthermore, the effects of hormone treatments upon human-derived bone cells will be investigated to provide mechanistic insights.

Rare Disease

The Effect of Prolonged Wheel Running on Muscle-Bone Properties in a Mouse Model of Moderate-Severe Dominant Osteogenesis Imperfecta

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Abstract

Background

Osteogenesis Imperfecta (OI) is a rare bone disorder caused by a mutation in COL1A1/1A2, which encodes collagen type I¹. Given the positive relationship between muscle and bone, muscle function deficits may contribute to bone mass deficits in those with OI². Studies have shown that exercise improves muscle function in OI, but the effect on the bone is unclear³. This study aims to determine the effect of voluntary wheel running (VWR) on muscle-bone parameters using the COL1A1^{Jrt/+} mouse model.

Methods

Four-week old, female wild-type and COL1A1^{Jrt/+} mice were divided into four groups: wild-type sedentary (WTS) (n=10), COL1A1^{Jrt/+} sedentary (CS) (n=8), wild-type VWR (WTVWR) (n=10), and COL1A1^{Jrt/+} VWR (CVWR) (n=8). Running wheels were blocked in sedentary groups, whereas the VWR groups had access for six-weeks. Forelimb grip force was assessed at five- and eleven-weeks old using a digital force gauge. Micro CT of the right femurs was performed to measure cortical thickness.

Results

Following the five-week protocol, the femoral cortical thickness of the VWR groups did not increase. Femoral cortical thickness of both wild-type groups was measured at 0.19 ± 0.02 mm and 0.16 ± 0.01 mm for the COL1A1^{Jrt/+} groups ($p < 0.001$). The relative grip muscle force (absolute force per body weight) for WTS was measured at 4.3 ± 0.5 g/g, compared to the WTVWR being 5.0 ± 1.1 g/g ($p < 0.05$). The relative grip muscle force for CS was 3.1 ± 0.6 g/g, while the CVWR mice was 3.1 ± 0.7 g/g ($p > 0.05$).

Conclusion

It was expected that exercise would have a positive effect on the muscle-bone parameters for the VWR mice. However, results indicated exercise had benefits on muscle, but no effect on bone thickness. The exercise in this study focused on one variable (VWR) and did not account for the duration or intensity of the activity. This study will aid in the development of recommendations to improve musculoskeletal health in youth with OI.

Description of hospital use by patients with osteogenesis imperfecta in the English NHS

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Abstract

Objective: To characterise the hospital use, stratified by age groups, of patients with osteogenesis imperfecta (OI) in the English National Health Service (NHS).

Methods: Routinely-collected aggregate data about all inpatient hospital admissions by patients with OI provided by Hospital Episode Statistics (HES) between April 1st 2014 and March 31st 2018 were used. Data were extracted on number of admissions (elective and non-elective), number of patients, length of stay, waiting time, and costs. The hospital use of OI patients was summarised using descriptive statistics.

Results: There were 16,245 hospital admissions reported for OI patients during the four years of analysis. The total cost for these admissions was £24,052,451. Of the 4,370 patients, 2,700 (62%) were females and the average number of admissions per patient per year was 3.3 for females and 4.4 for males. From all admissions, 54% were reported from the age groups between birth and 14 years of age (Figure 1). The shortest average length of stay per admission was 0.7 days for the age group five to nine years of age, and the longest was 10.5 days for the age group 90 to 94. The average cost was £1,260 for elective admissions, and £2,529 for non-elective admissions. The number of admissions increased on average by 2.1% per year whilst the average yearly increase in the number of patients was 6.4%.

Conclusions: NHS provides annually a large number of hospital admissions related to a high cost for patients with OI, which further increase over time with rates comparable to the whole NHS. The highest total number of admissions and total costs were reported for children below the age of 14, however the longer average length of stay and cost per admission were reported for older adults. Non-elective admissions were on average twice as expensive as elective admissions.

A cross-sectional study of patient-reported outcomes of individuals with X-linked hypophosphataemia within the UK

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Abstract

Objective

We described the patient-reported outcomes (PROs) of adults with X-linked hypophosphataemia (XLH) and examined if they are correlated.

Methods

Data were obtained from the RUDY study, which collects information from participants with rare diseases in the UK. Participant responses to nine instruments were extracted: EQ5D-5L and SF36 physical (PCS) and mental component score (MCS) as measures of quality of life, ESS and PSQI of sleep quality, PainDETECT and SF-MPQ-2 of pain, FACIT-F and FSS of fatigue, and HADS of depression and anxiety. Differences in mean scores between age groups and gender for first submitted instruments were tested using ANOVA and independent samples t-tests, respectively, and correlation between instruments examined via repeated measures correlation.

Results

The sample was comprised of 48 participants with XLH (77% females, median age 46 years). Mean scores were: EQ5D-5L=0.65, SF36-PCS=32.7, SF36-MCS=48.4, ESS=5.9 and PSQI=8.9, FSS=32.8 and FACIT-F=104.4, HADS-depression=4.7 and HADS-anxiety =6.2, SF-MPQ-2=1.9 and PainDETECT=9.3. Severe or extreme problems were reported in all instruments, with mobility (23%) and pain (23%) from EQ5D, and functional wellbeing (mean=18.2) and fatigue (mean=31.3) in FACIT-F being the most frequent. There was no statistically significant difference in mean scores by age group, or by gender except for PSQI, indicating that females (mean=9.6) experience a slightly poorer quality of sleep than males (mean=6.4). We found low to moderate correlation between instruments, few being statistically significant. The lowest correlation coefficient was between SF36-MCS and PainDETECT ($r=-0.019$) and highest between PSQI and FSS ($r=0.579$). The highest statistically significant correlations ($p<0.05$) were between SF36-MCS and FACIT-F ($r=0.513$).

Conclusions

People with XLH reported most severe problems in mobility, pain, functional well-being and fatigue. Mental health and fatigue reported the highest correlation. More research is needed to identify the PROMs that most consistently capture the main health problems and changes experienced by people with XLH.

Acknowledgement

We thank RUDY participants.

A longitudinal study of patient-reported outcomes of RUDY registry participants with X-linked hypophosphataemia in the UK

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Abstract

Objectives

To characterise the change over time in pain, sleep, fatigue, anxiety, depression and health-related quality of life (HRQoL) for adults with X-linked hypophosphataemia (XLH).

Methods

Data were collected from participants diagnosed with XLH and registered in RUDY, a cohort of individuals with rare diseases in the UK. Seven instruments were analysed: EQ5D-5L and SF36 physical component score (PCS) and mental component score (MCS) as measures of HRQoL, PSQI of sleep quality, PainDETECT and SF-MPQ-2 of pain severity, FACIT-F of fatigue, and HADS measuring depression and anxiety. Participants were invited to submit questionnaires every six months and we used data reported between July 2014 and August 2019 for the analysis. Change in mean scores over four time-points (up to two years) was estimated using mixed-effects linear regression models controlling for gender, age (at registry in RUDY) and time of follow-up.

Results

The sample included 48 RUDY participants with XLH, mostly female (77%) and with a median age of 46 years (range 19-85). SF-MPQ-2 and PSQI showed a slight improvement in mean scores whilst all other instruments reported fluctuating means. Mixed effects models revealed statistically significant ($p < 0.05$) time coefficients only for FACIT-F ($b = -2.135$, $p = 0.038$) and HADS-Anxiety ($b = 0.314$, $p = 0.031$), both reporting slightly worsening scores over time. Within FACIT-F, the functional well-being subscale was found to be the clear driver of change ($b = -0.754$, $p = 0.003$).

Conclusions

People with XLH report signals of deterioration in fatigue and anxiety over two years which could impact them significantly if sustained over longer periods of time. We did not find evidence of change in all other instruments, but individual-level trends suggest more research is needed to identify subgroups more likely to worsen as well as which patient characteristics may predict deterioration.

Acknowledgement

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Detecting Perthes Disease and Investigating the Effects of Aging on Hip Shape in Children

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Abstract

Objectives: This study investigated whether shape measures derived from radiographs could discriminate between children with healthy hips and those diagnosed with Perthes disease, a rare childhood hip condition. In addition, we investigated how age affects shape and the ability to discriminate.

Methods: We used 1,452 hip radiographs (1,348 healthy, 104 Perthes) collected from Alder Hey Children's Hospital. Ages ranged from 2-11 years (mean: 6.3, SD: 2.9). The proximal femur in every image was manually outlined using 58 points. Based on the latter, statistical shape models were constructed to quantify the radiographic bone shape variations using a small number of parameters. Our model extracted 11 shape parameters and explained 95% of the shape variation. We used gradient boosted trees (GBT), with an exponential loss, on these parameters to classify between healthy and Perthes.

Results: The discrimination ability was evaluated using 3-fold cross-validation (CV) experiments, ensuring to have a balanced age ratio across all folds. The classification performance was assessed using the mean receiver operating characteristic curve (ROC-AUC), precision, recall and F1-scores across all CV-folds. We obtained a mean ROC-AUC score of: 0.93 (+/- 0.03), precision: 0.72 (+/- 0.1), recall: 0.66 (+/- 0.18) and F1-Score: 0.68 (+/- 0.1). Our results show that the discrimination ability was affected by age. In younger children, the radiographic shape variation between Perthes and healthy hips is modest, but it increases in older children (see Figure). A flattened femoral head is a key feature of disease across all ages.

Conclusion: Radiographs of healthy and Perthes hips can be distinguished using shape parameters, with clearer discrimination in the older children. This demonstrates the potential of shape measures to help diagnose childhood hip diseases. It is likely that the classification performance could be further improved by adding more samples, particularly for diseased cases.

Regulation of TAZ by DEPTOR controls mesenchymal progenitors lineage commitment in response to PTH1R signaling

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Abstract

Skeletal elements are established from mesenchymal progenitors that form cartilage, which will be replaced by bone. These mesenchymal progenitors (or mesenchymal stem cells, MSCs) can differentiate into bone, cartilage and fat. Multiple signaling pathways are involved in lineage commitment such as BMPs, Hh and Wnt. Less studied is the role played by PTH/PTHrP; deletion of PTH1R in mice resulted in increased bone marrow fat. Two human skeletal dysplasias result from mutations in *PTH1R*; Jansen Metaphyseal Chondrodysplasia (JMC), which results from constitutive action of PTH1R and Blomstrand Chondrodysplasia (BOCD), caused by loss-of-function mutations. The specific molecular mechanisms by which these mutations mediate their effects remain unknown. Two key transcription factors (TF), RUNX2 and PPAR γ , drive MSCs differentiation into osteoblasts or adipocytes, respectively. TAZ, a member of the Hippo pathway, is a known regulator of these TF, coactivating and corepressing RUNX2 and PPAR γ respectively to promote bone formation and inhibit fat accumulation. We previously showed that DEPTOR, an mTOR inhibitor, accumulates in patients with JMC and demonstrated that PTH1R signaling controls DEPTOR degradation. Here, we show that JMC patients accumulate fat in the bone, whereas BOCD patients show chondrocytes immersed in the bone, indicating aberrant and distinct MSC differentiation caused by different mutations in the *PTH1R* receptor. DEPTOR directly interacts with TAZ; knockdown of DEPTOR in MSC cells results in a decrease in TAZ, RUNX2 and PPAR γ proteins following osteogenic induction. Moreover, TAZ and PPAR γ transcriptional activity are also decreased, consistent with decreased bone formation in BOCD patients. Knockdown of DEPTOR also increased SOX9 transcriptional activity, supporting increased chondrocyte formation in BOCD. Our results reveal a previously undescribed crosstalk between the mTOR component, DEPTOR, and the Hippo pathway effector, TAZ, and demonstrate their role in the regulation of mesenchymal progenitors lineage commitment in response to PTH1R signaling.

Characterization of comorbidity in X-linked hypophosphataemia: a prospective parallel cohort study using the UK CPRD

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Abstract

Objective: To describe comorbidity in patients with X-linked hypophosphataemia (XLH) and compare to general population controls without XLH.

Material and methods: The Clinical Practice Research Datalink (CPRD) GOLD was used to identify a cohort of XLH patients (1995-2016), along with a non-XLH cohort matched (1:4) on age, gender and GP practice at date of first XLH diagnosis (index date). Previously published phenotyping algorithms openly available from the online CALIBER portal were used to identify the first primary care diagnosis (and associated age) of 273 defined comorbid conditions during any eligible (i.e. up-to-standard) patient follow-up. For primary analysis, the individual conditions were merged into 15 major disease categories and the proportion of patients having ≥ 1 diagnosis in each category was compared between cohorts using univariable logistic regression. In secondary analysis individual conditions were compared. Only categories/conditions affecting $\geq 10\%$ of either cohort were included in these comparisons. Bonferroni corrected P-values were used.

Results: 64 patients graded likely or very likely XLH were included along with 256 patients without XLH. Of these, 45% were aged over 16 years at index date and more were female (70.5%). Recorded comorbidities (categorized) are presented in table. In secondary analyses of individual conditions, four were at least twice as likely to be present in XLH, but only depression met the Bonferroni threshold: odds ratio= 2.95 [95%CI: 1.47 to 5.92]; p=0.0023.

Condition category ¹	XLH cohort (n=64)			non-XLH cohort (n=256)			Odds Ratio			p-value ²
	Number with ≥ 1 condition	%	Mean age at earliest diagnosis	Number with ≥ 1 condition	%	Mean age at earliest diagnosis	Estimate	95% CI		
								Lower	Upper	
Circulatory/CV System	8	12.5	50.8	25	9.8	56	1.32	0.57	3.08	0.5211
Digestive System	10	15.6	45.8	49	19.1	36.5	0.78	0.37	1.64	0.5173
Endocrine System	10	15.6	38.8	13	5.1	49.5	3.46	1.44	8.31	0.0054*
Genitourinary System	15	23.4	43.1	49	19.1	41	1.29	0.67	2.49	0.4429
Mental Health Disorders	17	26.6	36.4	41	16	37.7	1.90	0.99	3.62	0.0527
Musculoskeletal Conditions	13	20.3	47.3	36	14.1	46.3	1.56	0.77	3.15	0.2169
Neurological Conditions	13	20.3	32.6	20	7.8	34.5	3.01	1.41	6.44	0.0046*
Respiratory System	21	32.8	26.7	63	24.6	22.2	1.50	0.83	2.71	0.1839
Skin Conditions	23	35.9	20.8	108	42.2	18.7	0.77	0.44	1.36	0.3639

¹ Only including categories affecting $\geq 10\%$ of either XLH cases or controls

² An asterix (*) indicates p-value was below the Bonferroni corrected threshold, which was p=0.0055556

Conclusion: Findings suggest XLH patients have elevated levels of comorbidity broadly defined as endocrinological and neurological, in addition to nearly three times the occurrence of depression, raising awareness of the multisystem effects of this rare bone disease.

A Natural History Study In Patients With ENPP1 Deficiency

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Abstract

Deficiency in ENPP1 activity, an enzyme that generates extracellular pyrophosphate (PPi) from ATP and modulates adenosine metabolism, results in life threatening disorders. Infants present with Generalized Arterial Calcification of Infancy (GACI), characterized by arterial calcifications and stenosis with approximately 50% mortality within the first 6 months of life. Surviving patients will develop Autosomal Recessive Hypophosphatemic Rickets type 2 (ARHR2).

To better characterize ENPP1 Deficiency, data was obtained from chart reviews of GACI and ARHR2 patients with mutations in *ENPP1* or *ABCC6* from the National Institutes of Health (NCT03478839) and Münster University Children's Hospital (NCT03758534).

Analysis revealed a diagnosis of GACI in 115 patients and ARHR2 in 36. Twenty-five survived GACI and later developed ARHR2. Of 109 probands with genetic confirmation, 88 had mutations in *ENPP1* and 21 in *ABCC6*. There were 25 of 76 patient deaths in the ENPP1 cohort. Consistent with past observations, arterial calcification (88%, 95%), joint calcification (64%, 33%), hypertension (65%, 67%) and short stature (stature-for-age less than third centile; 42%; 33%) were prevalent and consistent in ENPP1 and ABCC6 cohorts, respectively. Organ calcification (78%; 85%) cardiac dysfunction (78%, 85%), renal dysfunction (47%; 40%) and gastrointestinal dysfunction (63%; 58%) were also observed in both ENPP1 and ABCC6 cohorts, respectively. A higher prevalence of neurological complications was observed in the ABCC6 cohort (58% to 40%) while hearing loss (53%; 7%) and rickets (48%; 17%) was higher in the ENPP1 cohort.

This natural history study reveals the significant mortality and morbidity due to calcification of arteries and multiple organs, as well as organ dysfunction. Similar symptom prevalence was found in both ENPP1 and ABCC6 deficiencies suggesting that these disorders are founded in low PPi levels. Further analysis will further support and elucidate the progression of the disease.

Translational

Lumbar Spine Bone Mineral and Kinematics in Adolescent Cricket Fast Bowlers

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Abstract

Cricket fast bowling generates substantial tri-planar trunk movement and ground reaction forces (GRF), and bowlers show marked lumbar spine bone mineral adaptation. This study aimed to investigate whether lumbar bone mineral adaptation differs with GRF and range of lumbar extension, side flexion and lateral rotation at front foot contact. With institutional and NHS ethics approval, 19 adolescent male fast bowlers (mean \pm SD: 15.6 \pm 1.2 years; 180.0 \pm 6.8 cm; 69.4 \pm 7.7 kg) received an anterior-posterior lumbar spine DXA scan. Bone mineral content and density (BMC and BMD respectively) were derived for each lumbar vertebra and the lateral thirds of the non-dominant sides of each vertebra relative to bowling arm. A motion analysis camera system and two force plates recorded a maximal velocity trial for each participant. Retro-reflective markers were attached at the spinous processes of L5, L3, L1 and 5 cm bilaterally at the level of L2 and L4 to create upper and lower lumbar spine segments and produce angles in the sagittal, frontal and transverse planes at front foot contact. Correlations were sought between these variables, peak ground reaction force (GRF) and lumbar bone mineral, with height as a covariate. Contralateral rotation in L1-L3 significantly correlated to BMC at L1 and L5 ($r = 0.467$ to 0.469 ; $P \leq 0.044$), but this was no longer the case when height was included as covariate ($r = 0.273$ to 0.282 ; $P \geq 0.256$). Peak GRF significantly correlated to BMD and BMC on the non-dominant side of L5 both without ($r = 0.512$ to 0.488 ; $P \leq 0.034$) and with the covariate height included ($r = 0.533$ to 0.640 ; $P \leq 0.023$). Increased bone strain from muscular force and GRF during the bowling action, could increase adaptation at specific lumbar sites which could also be at an increased risk of bone stress injury.

Longitudinal effects of tibia loading on the bone morphometric, densitometric and mechanical properties in ovariectomized mice

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Abstract

Introduction: Tibia loading improves bone mass in mouse models of aging. However, it is unclear the longitudinal adaptation on bone structural and mechanical properties with oestrogen-deficiency. The aim of this study was to evaluate the longitudinal effects of mechanical loading on the morphometric, densitometric and mechanical properties of the tibia in the ovariectomized C57BL/6 mouse model of osteoporosis.

Methods: Six C57BL/6 mice were ovariectomized at 14-weeks-old and the right tibia *in vivo* microCT-scanned (10.4 μ m/voxel) at week 14 and every two weeks thereafter until 24 weeks of age. At age 19 and 21 weeks, compressive loading was applied to the right tibia and on alternate days (peak 12N, 40 cycles/day, 3 days/week). MicroCT images were rigidly registered to a reference tibia. Bone morphometry in the trabecular metaphysis and cortical midshaft, and total BMC along 80% of the tibia length, were measured. MicroCT images were converted into voxel-based homogeneous, linear elastic microFE models to estimate the bone stiffness under compression. Loading effects on bone properties (repeated measures ANOVA) and linear relationships between morphometry, BMC and bone stiffness were evaluated (Coefficient of Determination, R^2). The results were considered statistically significant where $p < 0.05$.

Results: With loading, a persistent increase in trabecular BV/TV, cortical thickness, total BMC and bone stiffness were observed (weeks 18-20, 20-22 Δ : +8-33%, $p < 0.05$, Fig. 1(a-c)). Stiffness was strongly related with cortical thickness and total BMC ($R^2 = 0.858-0.913$, $p < 0.001$, Fig. 1(d)), but not trabecular BV/TV ($R^2 = 0.196$, $p = 0.007$).

Discussion: Mechanical loading induced persistent improvements in trabecular BV/TV, cortical thickness, BMC and stiffness. However, findings were inconsistent with evidence for reduced stiffness after longer-term (6-weeks) treatment that is thought to damage existing tissue. This research highlights the utility of *in vivo* microCT-based microFE for non-destructive and longitudinal assessment of bone mechanics in preclinical mouse models of disease.

