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Clinical Update

The 4th Joint Meeting of ECTS and IBMS
Rotterdam, The Netherlands
25–28 April 2015

POSTMENOPAUSAL OSTEOPOROSIS, DIAGNOSTIC TOOLS, FRACTURE RISK ASSESSMENT AND TREATMENT

CU1.1
Update on Tools for Diagnosis and Monitoring Osteoporosis. DXA, TBS, VFA, Microindentation and Biomarkers.

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Dual energy X-ray absorptiometry (DXA) is currently the reference technique to diagnose osteoporosis, estimate fracture risk and monitor antiosteoporotic therapy. However, there are other determinants of bone strength that cannot be evaluated by only measuring bone mineral density (BMD), which are those related to bone quality such as the bone microarchitecture and bone tissue characteristics, among others. In addition, it is important to know the effect of different antiosteoporotic treatments in determinants of bone strength other than BMD, especially after long-term therapy. In recent years several diagnostic tools addressed to evaluate different aspects of bone quality have been developed, allowing better evaluation of bone strength. In this sense, the trabecular bone score (TBS), a texture parameter computed from DXA images, seems to analyse bone microarchitecture, thereby enhancing the bone assessment made by BMD measurement by adding the dimension of bone quality. In addition, DXA-assisted vertebral fracture assessment (VFA) is a useful method to detect the presence of vertebral fractures, an important and frequently overlooked risk factor for further fractures, allowing evaluation of BMD in the same session. Other methods such as the bone microindentation testing provides direct *in vivo* estimation of bone material strength measured in the cortical bone of the tibia Finally, although there are several discrepancies about the use of bone turnover markers in clinical practice, it should be noted that they can predict fracture risk and treatment-induced changes, accounting for a substantial proportion of fracture risk reduction. Nevertheless, controlling sources of variability and adopting international reference standards are necessary in this field. In summary, the integration of several new tools related to other aspects of bone quality may improve not only the identification of patients at high-risk of fracture but also the therapeutic approach and monitoring of these patients.

CU1.2
Abstract not available

CU1.3
Abstract not available

CU1.4
Abstract not available

CU1.5
Abstract not available

VITAMIN D – SKELETAL AND NON-SKELETAL EFFECTS

CU2.1
An Update on the Effects of Vitamin D on the Skeleton

Paul Lips
Dept Internal Medicine, VU University Medical Center, Amsterdam, the Netherlands

Vitamin D deficiency can cause secondary hyperparathyroidism and bone loss, mineralization defects, and in the long term rickets and osteomalacia. Vitamin D stimulates calcium and phosphate absorption from the gut, making these available for bone mineralization which mainly is a passive process. New laboratory observations show effects of vitamin D metabolites on osteoblast function and possibly mineralization. The prevalence of rickets is low in affluent countries but higher in some countries in Asia and Africa, and also in non-western immigrants. The global prevalence of osteomalacia depends on definition and can be estimated from biopsy and autopsy series, conservatively at about 1 %. Osteomalacia has been observed as a cause of hip fractures in the elderly. Vitamin D status is estimated according to serum 25-hydroxyvitamin D as deficient (<25 nmol/l) or insufficient (25-50 nmol/l) or adequate (>50 nmol/l). Low serum 25(OH)D (<50 nmol/l) has been observed in 50 to 80% of older persons, and in about 50% of all adults at least during winter. This results in a seasonal increase of parathyroid hormone and bone loss. The deficit of bone mineral density as a consequence of elevated PTH can be estimated at 1-2.5%. Clinical trials with vitamin D versus placebo in older persons have shown an increase of BMD of about 2%. Randomized clinical trials with vitamin D with or without calcium have shown a decrease of fracture incidence in 7 of 19 trials, no effect in 10 and an increase of fracture incidence in 2 trials. Most meta-analyses showed a decrease of fracture incidence with the combination of vitamin D and calcium, but no effect of vitamin D alone. The effect on fracture incidence could result from an increase of bone mineralization or a decrease of fall incidence.

CU2.2
Abstract not available

CU2.3
Abstract not available

CU2.4
Vitamin D and Cancer
David Feldman
Stanford, UK

Vitamin D is not really a vitamin but the precursor to the potent steroid hormone Calcitriol (1,25-dihydroxyvitamin D_3). Calcitriol, originally known for its regulation of calcium metabolism and bone homeostasis, has widespread extra-skeletal actions throughout the body including beneficial effects on the development and progression of cancer. The vitamin D receptor (VDR) is expressed in most tissues of the body. Most cancer data have been accumulated for colon, breast and prostate cancer but a benefit from vitamin D may be found in many cancers. Calcitriol regulates numerous extra-skeletal pathways that could play a role in determining cancer risk and prognosis including pathways involved in proliferation, apoptosis, differentiation, inflammation, invasion, angiogenesis and metastasis, and it therefore has the potential to affect cancer development and growth. Vitamin D status, which is determined by sunlight exposure, diet and supplements, might reduce the risk of developing cancer, and the appropriate regulation of cancer-relevant pathways by vitamin D might also have a place in the treatment of cancer. Multiple cell culture and animal models of cancer support a role for dietary vitamin D_3 and calcitriol in retarding cancer development and progression; however, data from human clinical trials are thus far inconsistent. Randomized control trials in humans do not yet exist to conclusively support a beneficial role for vitamin D, however accumulating results from preclinical and some clinical studies strongly suggest that vitamin D deficiency increases the risk of developing cancer and that avoiding deficiency and adding vitamin D supplements might be an economical and safe way to reduce cancer incidence and improve cancer prognosis and outcome. If adequate vitamin D concentrations do reduce risk, ensuring that people receive sufficient vitamin D would be an easily available, economical and safe modality to reduce cancer incidence and mortality.

CU2.5
Vitamin D in Pregnancy
Nicholas C Harvey
Southampton, UK

Low concentrations of 25-hydroxyvitamin D, the major circulating storage form, are common in the general population. Over recent decades, there has been increasing evidence for a role of vitamin D in disease pathogenesis far beyond the musculoskeletal system. Thus, many studies have investigated whether low levels of circulating 25-hydroxyvitamin D have a detrimental effect on pregnancy outcomes, for both mother and offspring, and whether supplementation with vitamin D might ameliorate such effects. We comprehensively surveyed this literature in a recent systematic review, funded by NIHR HTA. Suggestive positive associations were observed between maternal 25-hydroxyvitamin D concentration/ vitamin D supplementation during pregnancy, and offspring birthweight, serum calcium concentrations and bone mass, with some evidence for a protective effect of maternal 25-hydroxyvitamin D concentrations on pre-eclampsia. Overall, though, there was insufficient evidence to recommend vitamin D supplementation in pregnancy for any single health outcome. Such findings reinforce the need for high quality randomised control trials, such as the UK MAVIDOS Maternal Vitamin D Osteoporosis study, a multicentre, randomised, placebo-controlled, double-blind trial of 1000IU/day vitamin D3 (cholecalciferol) versus placebo from 14 weeks gestation till delivery of the offspring, in which the primary outcome is offspring DXA-measured bone mass, with pregnancy outcomes assessed as secondary endpoints. This study, which is currently in the analysis-phase, will test, in an interventional setting, earlier observations linking low maternal 25-hydroxyvitamin D concentration to reduced offspring bone mass, and gain valuable information regarding the role of vitamin D in pregnancy for other health outcomes. Such a rigorous interventional approach is essential to enable research questions to be adequately answered, such that alterations to public health policy maybe confidently based on robust evidence.

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COMPARATIVE ENDOCRINOLOGY OF CALCIUM REGULATION

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CECR 1

Which Came First: Parathyroid Hormone or Parathyroid Hormone-Related Protein?

Janine Danks
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Human chromosomes 11 and 12, which have parathyroid hormone (PTH) and parathyroid hormone-related protein (PTHrP) on their respective short arms, are known to have arisen through an ancient gene duplication event.[1] Every species from sharks through to man has at least one of each of the genes for PTH and PTHrP. In some of the fish species, multiple genes for both PTH and PTHrP have been identified. The phylogenetic analysis indicates that in the elephant shark (*Callorhinchus milii)* the point where the duplication resulting in these genes took place is close. There were two copies of the PTH gene in elephant sharks and these have not persisted in higher vertebrates indicating that one of these PTH genes has accumulated a number of deleterious mutations and has been lost in the process. The recent sequencing of the Japanese lamprey genome (*Lethenteron japonicum*)[2] is instructive. Jawless fish (lampreys and hagfish) have a pivotal position in evolutionary history, having undergone two whole genome duplications when compared with invertebrates. Like sharks, they have a cartilaginous skeleton but have the ability to move from seawater to freshwater. Whole genome duplications affect the animal's entire set of genes simultaneously and after the duplication one copy of the gene may be lost or acquire a new function. The Japanese lamprey genome database has been interrogated for the presence of *PTH* and *PTHrP*. Also tissues from the Japanese lamprey were examined for the expression of these genes. Certainly two receptors for PTH and PTHrP (*pth1r* and *pth2r*) are present in agnathan genome (*Petromyzon marinus)*.[3] Two PTH receptors have also been identified in invertebrates (*Ciona intestinalis*) but the ligands have not been found.[4] The evolutionary history of PTH and PTHrP might indicate which of these genes was the original gene and what possible novel roles each of the proteins/hormones may have.

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CECR 2

Calcium Regulation in Egg-Laying Chickens

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Chicken calcium metabolism in an egg-laying period is extraordinary when compared with all other classes of vertebrates, since the egg-laying hens produce up to three hundred eggs with hard-eggshell per a year. The eggshell consists of 5.7 g calcium carbonate ($CaCO_3$) containing about 2.3 g of net calcium. About 60-75% of the calcium in the eggshell is derived from dietary sources and the remaining of 25-40% from skeletal stores, called "medullary bone". Medullary bone is specifically developed in marrow cavities of long bones and plays an important role as a calcium reservoir for eggshell formation. There are osteoblasts, osteoclasts on medullary bone surface, as well as osteocytes embedded in the matrix. In the domestic hens, medullary bone formation and resorption occur alternately during the 24-hour egg-laying cycle. These cyclic changes in medullary bone metabolism depend on the site of an egg in the oviduct. Namely, when an egg is in the infundibulum, magnum and or isthmus of the oviduct, osteoblasts actively form medullary bone. On the other hand, when an egg enters into the shell gland and begins to be calcified, osteoclasts resorb medullary bone and mobilise calcium for eggshell formation. It has been reported that osteocytes play a key role for bone formation and resorption. However, the function of medullary bone osteocytes is still unknown. We have recently observed that carbonic anhydrase II (CAII) is localised in medullary bone osteocytes. CAII is a known proton generator which lyses calcified matrix, and is expressed in osteoclasts. In contrast to medullary bone osteocytes, cortical bone osteocytes do not contain carbonic anhydrase. These results suggest that osteocytes may also resorb medullary bone for eggshell formation.

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CECR 3

Abstract not available

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DEBATE ABSTRACTS

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D1.1

Abstract not available

D1.2

This House Believes that the Study of Mouse Physiology Usually Translates into New Insights into Human Physiology

Emily Sena
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The optimal use of animal models of human physiology requires at least three things; (1) that the animal studies are performed in such a way as to minimize the risks of bias; that is, that their findings accurately reflect what happened in the animal experiment; (2) that the animal model has some relevance to the human condition and that any limitations to their relevance are considered when such findings are interpreted; and (3) that all relevant information from animal models is available to those seeking new insights into human physiology. I will argue that, across a range of animal models of human physiology and pathophysiology, these conditions are not always met. Unless mouse physiology studies are a completely atypical beacon in a field characterized by poor reporting of measures to reduce the risk of bias (and I am aware of no empirical evidence to support this claim) then the prevalence of reporting of for instance randomization and blinding is likely to be low; and studies which, by virtue of this are at high risk of bias, are likely to overstate the effects observed. Further, the sheer volume of research in this field, publication bias, and the increasing pace of publication, make it more and more difficult for investigators to identify all relevant previous research as they plan new animal or human studies. Because of this, the narrative review article has become a less and less valuable method for synthesizing what is already know, and systematic approaches to such evidence synthesis are increasingly required. By increasing the validity of animal and human studies, and by adopting a systematic approach to evidence synthesis, we can increase the value of research which is done and reduce the waste that is a consequence of underpowered experiments, poor evidence synthesis, and high risks of bias in primary data.

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SYMPOSIA ABSTRACTS

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S1.1

Abstract not available

S1.2

Why Do We Need Clinical Trials?
Ian Reid
Auckland, New Zealand

Clinical trials have a number of characteristics that make them the most reliable way of assessing the efficacy and safety of medical interventions. Clinical trials use randomisation to produce groups which are comparable for both known and unknown factors influencing outcome

- Studies are blinded so that biases of participants or investigators cannot influence assessments of efficacy or safety
- Endpoints are pre-specified and consistently defined
- Analyses are planned a priori to prevent data dredging with a view to generating specific outcomes
- Studies are powered to be able to detect a clinically meaningful difference in endpoints

These safeguards allow a high level of confidence in the reliability of any statistically significant difference found between the treatment groups. No other form of clinical investigation has comparable rigour. Observational studies have a role to play in hypothesis generation and in monitoring events too rare to be assessed in trials. The repeated instances of major discrepancies between trial outcomes and analyses of observational data reinforce the belief that all major treatment policies in medicine should be based on clinical trial results.

S2.1

New Directions in Cancer and Bone - New Treatments
Robert Coleman
University of Sheffield, UK

Over recent years the introduction of bone-targeted treatments has transformed the clinical care of patients with metastatic bone disease. Both bisphosphonates (BP) and denosumab have a major beneficial impact on skeletal morbidity, leading to improved quality of life and physical functioning and reduced demands on expensive interventions and hospital care. Improvements have also been seen in radiation based therapies with the widespread use of single large (8Gy) treatment fractions for bone pain, improved delivery of high dose radical radiation treatment to oligo-metastatic disease through the use of stereotactic ablative radiation (SABR) and the introduction of highly effective and safe, novel radiopharmaceuticals such as radium-223. Coupled with these improvements in bone-targeted treatments, are rapid developments in both cytotoxic therapy and molecularly targeted treatments for cancer. These can now provide disease control, albeit only temporary at present, to most patients and are resulting in steadily improved survival and ever increasing treatment options such that, for many, advanced cancer is becoming a chronic disease and no longer the rapid death sentence of yesteryear. Some of these targeted cancer treatments also have important effects on bone metabolism that are of importance to bone specialists. Finally, improved surgical and interventional radiologic options are contributing to the multidisciplinary care of >1 million patients with metastatic disease worldwide. Through their profound effects on bone physiology, bone targeted treatments can potentially modify the process of metastasis and have important effects on disease outcomes as well as bone health. Metastasis prevention trials have reported variable outcomes in terms of disease recurrence with efficacy apparently influenced by tumour type and, at least in breast cancer, by levels of reproductive hormones. At least in breast cancer we now understand better the potential of BP to influence relapse and survival rates. In a recent initiative with the Early Breast Cancer Clinical Trials Group, we sought individual patient data for meta-analysis from 36 randomised trials that compared BP to no BP (placebo or open control) and evaluated the effect of adjuvant BP on disease outcomes. Data on 18,766 women were received, with 3,453 and 2.106 breast cancer recurrences and deaths respectively. BPs reduced first distant recurrence in bone (RR=0.83; 95%CI 0.73-0.94, 2p=0.004) but not recurrence at other distant sites, or local or contralateral breast cancer recurrence. Importantly, there was a significant interaction between treatment efficacy and menopausal status with no apparent benefit for premenopausal women but highly significant reductions for postmenopausal women in bone recurrence (RR=0.72; 95%CI 0.60-0.86, 2p=0.0002) and breast cancer mortality (0.82; 95%CI 0.73-0.93, 2p=0.002). These findings are changing clinical practice and are being followed by adjuvant trials of other agents such as denosumab and in other disease settings such as prostate and lung cancers to see if we can build further on these exciting clinical observations and fulfill the preclinical promise of the past 25 years.

S2.2

Lysyl-oxidase and the Pre-metastatic Bone Niche

Alison Gartland, Thomas R. Cox, Robin M.H. Rumney, Erwin M. Schoof, Lara Perryman, Anette M. Høye, Ankita Agrawal, Demelza Bird, Norain Ab Latif, Hamish Forrest, Holly R. Evans, Iain D Huggins, Georgina Lang, Rune Linding and Janine T. Erler
Sheffield, UK

Lysyl oxidase (LOX) is an exciting new potential therapeutic target for the treatment and prevention of metastatic disease. We and others have shown that LOX is highly expressed by invasive/metastatic cancer cells, enhances tumour progression and that patients with high LOX expression had lower metastasis-free survival. We have also shown that LOX is critical for pre-metastatic niche formation in soft-tissue (lungs, liver and brain) enhancing bone marrow-derived cell invasion and thereby enabling colonisation of metastasising tumour cells. More recently we have begun to unravel the role of LOX in the bone pre-metastatic niche, in particular the early events governing osteolytic lesion formation. Using multiple *in vitro* and *in vivo* models, and a large clinical cohort, we show that LOX gene expression is significantly correlated with osteotropism and bone relapse. We show that high expression of LOX in primary breast tumours or systemic delivery of LOX *in vivo* leads to osteolytic lesion formation, and that silencing or inhibition of LOX activity abrogates this. The enzymatic activity of tumour-secreted LOX affects both osteoclasts and osteoblasts, disrupting normal bone homeostasis leading to bone lesion formation. These changes and lesions occur prior to tumour cell arrival in the bone and act to provide the initial foothold for circulating tumour cells to colonise the niche and form bone metastases. Mechanistically, we identify tumour-secreted LOX as a novel regulator of osteoclastogenesis through NFATc1 transcription factor translocation. In summary, we have uncovered a novel step in bone metastasis and mechanism of bone homeostatic regulation, opening up new opportunities for therapeutic intervention with important clinical implications.

S3.1

Organoid Cultures for *In-vitro* Disease Modeling

Marc van de Wetering
The Netherlands

The intestinal epithelium is the most rapidly self-renewing tissue in adult mammals. We originally defined Lgr5 as a Wnt target gene, transcribed in colon cancer cells. Two knock-in alleles revealed exclusive expression of Lgr5 in cycling, columnar cells at the crypt base. Using lineage tracing experiments in adult mice, we found that these Lgr5+ve crypt base columnar cells (CBC) generated all epithelial lineages throughout life, implying that they represent the stem cell of the small intestine and colon. Lgr5 was subsequently found to represent an exquisitely specific and almost 'generic' marker for stem cells, including in hair follicles, kidney, liver, mammary gland, inner ear, tongue and stomach epithelium. Single sorted Lgr5+ve stem cells can initiate ever-expanding crypt-villus organoids, or so called 'mini-guts' in 3D culture. The technology is based on the observation that Lgr5 is the receptor for a potent stem cell growth factor, R-spondin. Similar 3D cultures systems have been developed for the Lgr5+ve stem cells of stomach, liver, pancreas and kidney. Using CRISPR/Cas9 technology, the CFTR locus has been corrected in intestinal organoids of cystic fibrosis patients.

S3.2

New Strategies for Bone Repair – Harnessing Skeletal Stem Cells From Bench to Clinic

Richard OC Oreffo
Bone and Joint Research Group, Centre for Human Development, Stem Cells and Regeneration, Institute of Developmental Sciences, University of Southampton, Southampton, UK

Skeletal stem cells confer to bone its innate capacity for regeneration and repair. Bone regeneration strategies seek to harness and enhance this regenerative capacity to repair skeletal defects resulting from trauma and disease with the application of cells, typically isolated from the patients themselves, in combination with porous biomaterials or scaffolds. Skeletal stem cells, commonly referred to as Mesenchymal stem cells, or human bone marrow stromal stem cells are defined as multipotent progenitor cells with the ability to generate cartilage, bone, muscle, tendon, ligament and fat. To date, technologies to facilitate the identification and isolation of specific skeletal stem cells and development of scaffolds that address issues of growth factor delivery and angiogenic support to aid de novo tissue formation remains a significant unmet clinical need. We have developed protocols for the isolation, expansion and translational application of skeletal stem cell populations with cues from developmental biology, nanotopography and nanoscale architecture as well as biomimetic niche development informing our skeletal tissue engineering approaches. We have developed ex vivo approaches to bone formation evaluation and analysis and central are large animal in vivo translational studies to examine the efficacy of skeletal stem and cell populations in innovative scaffold compositions for orthopaedics. This talk will also highlight current clinical translational studies to examine the efficacy of skeletal populations for orthopaedic application. Advances in our understanding of skeletal stem cells and their role in bone development and repair, offer the potential to open new frontiers in bone regeneration and offer exciting opportunities to improve the quality of life of many.

S4.1

Modelling and Remodelling of the Corticol Bone

David W. Dempster
New York, USA

Cortical bone comprises 80% of the adult skeleton. Yet in recent years it has received less attention from bone researchers than cancellous bone. Cortical bone undergoes both modeling (resorption and formation occurring independently of each other at different sites) and remodeling (coupled and sequential resorption and formation occurring at the same site). Modeling and remodeling occur on both the endocortical and periosteal surfaces of the cortex, whereas within the cortex only remodeling – referred to as Haversian remodeling - takes

place. Bone modeling plays a key role during growth and determines the size and shape of the adult bone. This process is largely under genetic control. Disruptions in the modeling process during growth results in deformed bones, the classic case of which is the Erlenmeyer flask deformity. Modeling also occurs in adults, primarily in response to changes in mechanical loading. For example, modeling is responsible for the increase in mass and strength of the forearm bones in the dominant arm of experienced tennis players. Remodeling of the cortex, occurs, throughout life and is influenced by a variety of endocrine, mechanical and local factors. After skeletal maturity, remodeling increases with age and particularly after menopause. On the endocortical surface net resorption exceeds formation leading to expansion of the medullary cavity, thinning and trabecularization of the cortex. On the periosteum, net formation exceeds resorption leading to a gradual increase in the diameter of the bone, but the rate of periosteal expansion is less than that of the endocortex and so cortical thickness declines. Resorption also exceeds formation in the intracortical envelope leading to an increase in the diameter of the Haversian canals and evolving Haversian systems can fuse to form "giant" canals. The net result of these age- and menopause- related changes is loss of cortical, mass, structure and strength leading to skeletal fragility.

S4.2

The Effect of Osteoporosis Treatments on Cortical Bone: Insights from Clinical Trials

Juliet Compston
Cambridge, UK

In clinical trials conducted in postmenopausal women with osteoporosis, reductions in fracture risk of up to 70% in the spine, 40% in the hip and 15-20% at non-hip non-vertebral sites have been demonstrated. The limited efficacy at non-vertebral sites is a concern, given the high burden and cost of these fractures. Whilst poor adherence to therapy and continuing falls risk are likely to contribute to the small effect on non-vertebral fractures, drug-specific factors may also operate. Investigation of approved and investigational drugs with differing mechanisms of action, together with improved methods for studying cortical bone structure has provided some new insights into mechanisms by which drugs may influence cortical bone strength. A number of limitations in the assessment of cortical bone structure and strength should be recognised. First, effects of drugs on cortical bone may vary according to skeletal location. Secondly, even at a particular site, for example the femoral head and neck, there is marked heterogeneity of structure and strength and effects of drugs may be focal. Thirdly, many of the current methods for in vivo assessment of cortical bone are limited by inadequate resolution leading to difficulties in accurately assessing cortical porosity. Finally, other characteristics of cortical bone, for example the degree of mineralization and its homogeneity, and the structure of the mineral/matrix composite may affect estimates of cortical bone structure and strength. Notwithstanding these limitations, clinical trials of approved and investigational drugs have indicated interesting differences in effects on cortical bone. Whilst bisphosphonates reduce or prevent age-related deterioration of cortical bone structure and strength, denosumab appears to increase cortical thickness, perhaps as a result of its greater accessibility to intracortical bone. The effect of parathyroid hormone peptides is variable and influenced by mechanical loading. Cathepsin K inhibitors and sclerostin inhibitors, which uncouple bone remodeling by distinct mechanisms may have greater osteoanabolic effects on cortical bone although whether this translates into greater efficacy in reducing non-vertebral fractures remains to be established.

S5.1

Abstract not available

S5.2

Subchondral Bone as a Source of Arthritis Pain

David A Walsh
UK

Arthritis is a major source of chronic pain, distress and disability, although mechanisms of arthritis pain are incompletely understood, and current treatments often have limited efficacy. In osteoarthritis (OA), subchondral bone marrow lesions (BMLs) seen by magnetic resonance imaging are associated with current pain, and change in parallel with changing pain. Bone marrow lesions reflect diverse histological characteristics, with increases in bone turnover, as well as fibrovascular and inflammatory cell infiltration of bone marrow spaces. These changes are associated with increased osteoclast activity. Interventions that reduce osteoclast activity display analgesic efficacy in OA, both in animal models and in clinical trials. Bisphosphonates reduced pain in people with BMLs in a randomized clinical trial. Osteoprotegerin-Fc reduced pain behaviour in rodent models of OA. Osteoclasts, by reducing pH within the subchondral bone, might facilitate nociceptor activation through pH sensitive ion channels such as TRPV1. Furthermore, osteoclasts express factors that might sensitise nociceptors, and release other pain mediators during matrix degradation. In the medium to long term, structural changes at the osteochondral junction might further contribute to OA pain. The tidemark (junction between calcified and non-calcified articular cartilage) forms a barrier to diffusion and mass transport of molecules from within the synovial cavity into subchondral bone. In OA, this barrier is disrupted, initially by the penetration of vascular channels from subchondral bone spaces into the non-calcified cartilage, and later through fissuring or cleavage of the articular surface leading to microscopic or macroscopic chondral defects. Osteochondral defects are further associated with bone marrow lesions and articular pain. Far from being a passive, degenerative disease, OA is associated with increased metabolic activity and growth in subchondral bone. Sensory nerve growth from subchondral bone leads to innervation of vascular channels within the non-calcified cartilage, and so a tissue that is normally not innervated might become a source of OA pain. BMLs and increased osteoclast activity are also observed in rheumatoid arthritis, especially in late stage disease. Novel pharmacological interventions might complement joint replacement surgery as a means of reducing arthritis pain originating in subchondral bone.

S6.1

Abstract not available

S6.2

Treatment of Osteoarthritis: Present and Future

João Eurico Fonseca
Lisbon Academic Medical Centre, Lisbon, Portugal

During this presentation the cornerstone of current osteoarthritis treatment will be reviewed, including critical appraisal of the evidence of presently used drugs, integrating them with available guidelines. In addition, new concepts of osteoarthritis treatment interventions, interfering with metabolic and inflammatory pathways and with structure and biomechanics will be discussed.

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WORKSHOP ABSTRACTS

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W1.1
Decellularised Tissues

Shu Barylak
USA

The use of biologic scaffolds composed of extracellular matrix has found widespread use in multiple body systems including the musculoskeletal system, gastrointestinal tract, lower urinary tract, central nervous system, and cardio-vascular system, among others. Such ECM scaffold materials are prepared by decellularization of source tissues. The mechanism by which these inductive scaffold tissues facilitate constructive tissue remodeling include recruitment of endogenous stem cells, modulation of the host innate immune response, and the temporary mechanical substrate for tissue reconstruction. The presentation will include methods of scaffold preparation, mechanisms of action, and clinical applications with a focus upon musculoskeletal repair.

W1.2
Talking to Cells: Surface Topography as Tool to Evoke Cellular Responses

Jan de Boer
Laboratory of Cell Biology-inspired Tissue Engineering, MerIn Institute, Maastricht University, The Netherlands

Research in our laboratory is dedicated to understanding and applying basic cell biological principles in the field of biomedical engineering, in particular in the regeneration of bone tissue. The research program is characterized by a holistic approach to both discovery and application, aiming at combining high throughput technologies, computational modeling and experimental cell biology to streamline the wealth of biological knowledge to real clinical applications. In my seminar I will present our latest work on controlling the interaction of cells with biomaterials through design of surface topography. For instance, we are interested in the bone-inducing properties of a subset of porous calcium phosphate ceramics and show how through reverse engineering, we are uncovering an interesting and complex response of cells to materials. Inspired by this, we have started to design high throughput screening strategies of biomaterials libraries, and in particular libraries of surface topographies. Using a design algorithm, we have generated numerous different patterns, which can first be reproduced on a silicon mold and then imprinted onto polymers using microfabrication. After cell seeding, we use quantitative high content imaging and machine learning algorithms to characterize the response of the cells to the thousands of different surfaces and learn more about the relation between surface topography and cell response. For instance, we have identified surfaces which stimulate osteogenic differentiation of mesenchymal stem cells and we are currently testing whether these surfaces can be applied in orthopedic surgery.

W1.3
Engineering of 3D Vascularized Human Tissues

Heike Walles
Germany

Regenerative Medicine is a multidisciplinary field that combines engineering, physical and biological sciences and medicine with the overall goal to restore or to replace damaged tissues or organs. In order to reach this goal there are several strategies such as the application of cell suspensions or biomaterials. Furthermore the use of tissue engineering constructs, which are produced by a combination of both cells and scaffolds, is a more sophisticated approach. Therefore, it is necessary to isolate cells from the patient that are cultured together with the scaffold in vitro before this construct can be implanted into the patient. Vascularization is a major challenge in creating tissues *ex vivo*. Complex tissue engineered constructs exceeding a thickness of 100-200 µm need a vascular system in order to supply the cells with oxygen and nutrients and moreover remove waste products. This restricts generation of tissues with an appropriate size for clinical application and complex tissues such as the bone. We developed 3D vascularized tissues based on decellularized porcine small bowl segments and preserved tubular structures of the capillary network within the collagen matrix which is functional associated with one small vein and artery (biological vascularized scaffold - BioVaSc). This vascularized matrix enables the generation of a functional artificial vascular network and vascularized tissues as trachea, bone, skin, fatty tissue, intestine and liver. Possible application of this technology is the so called ATMPs - advanced therapy **medicinal** products. We are in preclinical and clinical testing of different vascularized implants. During the talk will be shown that it is possible to use the BioVaSc platform technology to generate autologous human transplants wich can be connected to the recipient vascularization. An overview of the initiated phase I/II clinical TraVaSc trial (tracheal reconstruction), the preclinical trial for the treatment of critical bone size defect (BoneVaSc) and the application as SkinVaSc and AdiVaSc will be shown. At the end of the presentation an overview of non-destructive methods to characterize the complex implants will be given. Our Team is focusing on the impedance and Raman spectroscopy for these applications.

W2.1

Abstract not available

W2.2

Abstract not available

W2.3

Pharmacological Management of Sarcopenia

William Evans
Muscle and Health Division, KineMed, Emeryville, CA and Division of Geriatrics, Duke University, Durham, NC

Sarcopenia is a life-long process of loss of muscle mass and strength. The causes of sarcopenia are complex and multifactorial. Among the many factors involved are insulin resistance, reduced physical activity, decreased dietary protein intake along with an age-related increase in dietary protein needs, loss of motor units, and decreased anabolic factors such as IGF, testosterone, and an increase in myostatin levels. Exercise has a profoundly positive effect even in very old and frail people. In particular, resistance exercise results in a large increase in strength, improved balance, increased bone density, and improved functional capacity. However, extreme loss of muscle mass and strength occurs in many older people during hospitalization and bed rest. In addition, muscle wasting associated with chronic disease (such as CHF, COPD, Cancer, and CKD) in elderly people may accelerate sarcopenia. Under these circumstances exercise and diet may play a very limited role. A new generation of anabolic therapies may rescue elderly people from conditions of rapid muscle wasting and improve functional capacity sufficiently to allow elderly people who suffering muscle wasting to recover more rapidly and decrease the risk of disability. These therapies include Selective Androgen Receptor Modulators, TGF-beta superfamily targets such as anti-myostatin anti bodies, ActRIIb, FLRG, and ghrelin. These anabolic agents stimulate the rate of muscle protein synthesis by activating mTOR in muscle. Protein synthetic pathways appear to be the most promising targets rather than anti-catabolic therapies. In addition, most of the drugs that stimulate muscle protein synthesis have been demonstrated to also increase bone density. Data will be presented on preclinical and clinical trials, with particular attention of aging.

W3.1

Regulation of Bone Remodelling by Semaphorins and Sensor Innervations

Shu Takeda
Japan

It was believed that cytokines and hormones are main regulators of bone remodeling. However, this view has been challenged. Organ network has been shown to play a major role in homeostasis, recently. Bone is not the exception. Clinically, it is well known that head trauma accelerates fracture healing. Advances in molecular genetics revealed that neurons and neuropeptides, including sympathetic nervous system, are intimately involved in bone remodeling. Semaphorin 3A (Sema3A) is a diffusible axonal chemorepellent that plays an important role in axon guidance. Previous studies have demonstrated that Sema3A is an osteo-anabolic autocrine and, accordingly, Sema3A-KO mice develop a low bone mass due to decreased bone formation. However, recently, we demonstrated that mice lacking Sema3A in neurons had low bone mass similar to Sema3A-KO mice, indicating that neuron-derived Sema3A is responsible for the bone abnormalities independent of the local effect of Sema3A in bone. Indeed, sensory innervations of trabecular bone were significantly decreased in neuron-specific Sema3A-KO. Moreover, ablating sensory nerves decreased bone mass in wild-type mice, whereas it did not deteriorate low bone mass phenotype in neuron specific Sema3A-KO mice, further indicating the essential role of sensory nervous system in normal bone homeostasis. Thus, we demonstrated that sensory nervous system is also a critical regulator of bone remodeling.

W3.2

Control of Bone Metastases by the Sympathetic Nervous System

Florent Elefteriou
USA

We have shown that activation of the sympathetic nervous system (SNS) alters the bone marrow environment and causes bone loss in mice, via some of the same signaling molecules that have been implicated in breast cancer metastasis to bone. In addition, chronically depressed patients do not seem to benefit from newly developed treatments for cancer and present with shorter survival. Because severe depression and chronic stress stimulate sympathetic outflow, we hypothesized that SNS activation induced by psychosocial factors remodels the bone marrow environment to make it a fertile “soil” for breast cancer cells, thereby promoting disseminating cancer cell establishment in the skeleton and leading to reduced survival. A series of experiments in preclinical models of bone metastasis supported this hypothesis, identified RANKL as a SNS-induced cytokine promoting breast cancer cell migration and establishment in bone, and showed that the beta-blocker propranolol could reduce the skeletal dissemination of cancer cells. Retrospective clinical studies also suggest a beneficial effect of sympathetic blockade in term of less advanced disease at diagnosis, lower cancer-specific mortality, longer disease-free survival and reduced metastasis development and tumor recurrence, particularly in patients that have taken beta-blockers before diagnosis. Therefore, beta-blockers or therapies normalizing sympathetic tone might be beneficial as early adjuvant therapies to limit skeletal metastases and to improve prognosis in patients with breast cancer.

W3.3

Hypothalamic Control of Bone and Fat

Paul A Baldock
Australia

Skeletal research is currently undergoing a period of marked expansion. One aspect in particular is the relationship between bone and fat metabolism. Emerging evidence indicates that bone and adipose activity are co-regulated and inter-dependent. Signals from fat cells are known to regulate bone mass, with prominent adipokines such as leptin and adiponectin. One bone/ fat-active pathway is controlled by neuropeptide Y

(NPY), most prominently in the hypothalamus, but also in the osteoblast. NPY is a critical downstream mediator of leptin actions, and is fundamental to the skeletal response resulting from hypothalamic leptin signaling. Elevation in central NPY expression produces powerful stimulation of appetite and adipose tissue production, but also inhibition of osteoblast activity, through neural relays from the brain to the bone. Interestingly, signals produced by bone cells are now being identified that are capable of regulating fat cells, both directly and through central hypothalamic signaling. Osteocalcin, a protein secreted by osteoblasts, is capable of regulating of energy and glucose homeostasis, reducing fat mass and increase insulin sensitivity. We have recently, identified a novel central loop for osteocalcin signalling to regulate bone, but also capable of regulating adipose and glucose homeostasis. In conclusion, the link between energy and bone homeostasis is far more complex than previously appreciated, with multiple axes of control, involving both central and direct signalling pathways. These signalling axes reveal a classic, hypothalamic pattern of feedback and efferent neural/endocrine control.

W4.1
Nanomedicine: What is it?

Robert Chapman, Molly M. Stevens
London, UK

The ability to engineer the structure of materials on the nanoscale has enabled access to extraordinary optical properties, the compartmentalisation and release of molecular components on demand, and the ability to interact with biological systems in unprecedented ways [1-2]. The design and use of these materials to produce a medical effect, a field known as nanomedicine, is poised to have a profound impact on healthcare [3]. The power of nanomedicine can be observed in a number of ways. The unique optical properties of inorganic nanoparticles and quantum dots have enabled the development of a range of new biosensors for diagnosis of disease. By linking small changes in the surface chemistry to dramatic and visible changes in bulk properties, the ease and sensitivity of detection of a range of protein and nucleic acid biomarkers has been greatly improved. Nanoparticles have also enabled new bioimaging techniques, through their ability to enhance Raman scattering allowing rapid imaging of chemical information within cells, and their use as targeted imaging agents *in vivo*. Patterning of surfaces with nanoscale precision has allowed the investigation of biological interfaces and processes *in vitro*. Nanomaterials that can compartmentalise molecular components, such as liposomes, exosomes and self-assembled polymeric nanoparticles have also been widely exploited as drug delivery vehicles. Targeting these materials towards particular receptors and linking their properties to external triggers such as light, pH, temperature or enzymatic activity, has enabled the delivery of therapeutics to particular locations. This talk will provide an overview of the field of nanomedicine and discuss the use of nanoparticles in biosensing, biomedical imaging, diagnostics and therapy, with examples from our work.

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W4.2
Molecular Imaging Using Nanoparticles

Fabian Kiessling
Germany

Nanoparticles are frequently used as carriers for therapeutic drugs but can also be used as part of imaging compounds for various imaging modalities. In this talk, an overview will be given on the use of nanoparticles for imaging and theranostics. Most nanoparticles are characterized by either long blood half-lifes or by rapid uptake by the RES. In addition, they tend to selectively accumulate in tissues with high vessel leakiness due to EPR effects. Thus, they are favourably suited as drug carriers in oncology. The large surface of nanoparticles enables to generate different functionalities in the same probe and adding an imaging marker to such a therapeutic probe results in a theranostic agent that can be used for patient selection and for controlling probe accumulation. Another important indication for diagnostic nanoparticles is imaging of the RES as it is used clinically to detect tumors in liver and lymph nodes using iron oxide nanoparticles. Since nanoparticles are internalized strongly by cells they can also be used for cell labelling and in vivo cell tracking experiments. Alternatively, in the emerging field of tissue engineering nanoparticles can be used to label scaffolds in order to localize the transplants and to monitor their resorption and remodelling. For targeted imaging, however, except for very small renally cleared nanoparticles, nanoparticles are critical since their penetration into tissues is low and high background signal is generated due to EPR effects. If targeted nanoparticles are designed for intravascular imaging, however, one may consider designing them larger (up to micrometer size) in order to avoid the unspecific retention in the interstitial space. In summary, although there are clear indications for the use of nanoparticles as diagnostic probes they are no magic bullets for imaging purposes and their use should be carefully considered taking into account alternative strategies such as the use of small molecules.

W4.3
Targeted Nanomedicine and Nanotheranostics

Gert Storm
Dept. Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences (UIPS), Utrecht University, Utrecht, The Netherlands. University Medical Centre Utrecht (UMCU), Utrecht, The Netherlands. Dept. Controlled Drug Delivery, MIRA Institute for Biomedical Technology and Technical Medicine, University of Twente, Enschede, The Netherlands

Since the introduction of the liposomal doxorubicin formulation Doxil®/Caelyx® on the market 20 years ago, a number of targeted nanomedicine formulations have become part of treatment regimens in the clinic. An update will be given of problems encountered and current strategies to tackle them. The preclinical use of nanomedicine formulations for thera-

peutic and diagnostic applications is increasing exponentially. Many different systems and strategies have been developed for drug targeting to pathological sites, as well as for visualizing and quantifying important (patho-) physiological processes. In addition, ever more efforts have been undertaken to combine diagnostic and therapeutic properties within a single nanomedicine formulation. These so-called nanotheranostics are aimed to provide valuable information on drug delivery, drug release and drug efficacy, and they are considered to be highly useful for personalizing nanomedicine-based (chemo-) therapeutic interventions.

W5.1

Next Generation Sequencing

Uwe Kornak
Germany

Next generation sequencing (NGS) has begun to dramatically change research and diagnostic approaches. Compared to conventional Sanger sequencing the price per sequenced base has dropped several thousand-fold leading to a shift of the bottleneck from sequence data generation to bioinformatic interpretation. After an introduction into the principles of NGS different research applications centered on gene regulation and expression signatures will be discussed. Next, the upcoming role in the clinics will be highlighted. The possibility of parallel sequencing of hundreds of genes up to the whole exome in targeted gene panel approaches or even whole genome sequencing has shifted the paradigm in differential diagnosis of rare skeletal disorders to a "sequence first" strategy. But also in seemingly common skeletal disorders the parallel sequencing of many candidate genes offers unprecedented insight into the underlying disease etiologies. In a pilot study on early onset osteoporosis we found evidence for monogenic inheritance in a significant portion of patients. With increasing numbers of sequenced patients and appropriate available databases probably also more complex oligogenic inheritance patterns can be elucidated. Given the relevance of this genetic information for personalized prognosis and treatment it is to be expected that increasingly rapid genotyping at dropping costs will become a standard tool in many fields of medicine.

W5.2

Systems Dissection of Pluripotency

Frank Buchholz
Dresden, Germany

Pluripotent stem cells are important to understand early mammalian development and hold great promise for future regenerative therapies. The identification of factors required to maintain pluripotency is a pivotal step to fully develop these cells for applied purposes and to decipher embryonic differentiation. I will describe the combined use of genome-scale RNAi screens targeting protein coding and long non-coding RNAs with genetic interaction, protein localization and protein-level dependency studies to delineate connectivity between factors that control ES and EpiSC identity. Examples of newly identified protein coding, and long non-coding RNAs that impact on pluripotency will be presented.

W5.3

Abstract not available

W6.1

The Evaluation of Bone Quality in Healthy and Diseased Human Cortical Bone

Robert O. Ritchie
USA

As one of the most important natural materials, cortical bone is a composite material comprising assemblies of tropocollagen molecules and nanoscale hydroxyapatite mineral crystals, forming an extremely tough, yet lightweight, adaptive and multi-functional material. Bone has evolved to provide structural support to organisms, and therefore its mechanical properties are vital physiologically. Like many mineralized tissues, bone can resist deformation and fracture from the nature of its hierarchical structure, which spans molecular to macroscopic length-scales. In fact, bone derives its fracture resistance with a multitude of deformation and toughening mechanisms that are active at most of these dimensions. Here we examine ways to quantity the "quality" of bone in terms of its basic mechanical properties of strength, ductility and most importantly resistance to fracture (toughness). We show that bone's strength and ductility originates primarily at the scale of the nano to submicron structure of its mineralized collagen fibrils and fibers, whereas bone toughness is additionally generated at much larger, micro- to near-millimeter, scales from crack-tip shielding associated with interactions between the crack path and the microstructure. We further how the effectiveness with which bone's structural features can resist fracture at small to large length-scales can become degraded by biological factors such as aging and disease, which affect such features as the collagen cross-linking environment, the homogeneity of mineralization, and the density of the osteonal structures. In this regard, we specifically examine the effects of various diseases, such as vitamin D deficiency, *osteogenesis imperfecta* and Paget's disease, on bone quality, and present the results of preliminary experiments on the effects of bisphosphonate treatments as a possible cause of atypical femoral fractures.

W6.2

Bone Fragility Beyond Bone Strength: The Clinical Hip Fracture as A Challenge to Basic Understanding

Jonathan Reeve
Oxford, UK

Every hip fracture begins with a microscopic crack that enlarges explosively. Most hip fractures in the elderly occur on falling from standing height, usually sideways or backwards. The typically moderate level of trauma very rarely causes fracture in younger people. This growing fragility may follow the decline of multiple protective mechanisms at many length scales from nanometres to that of the whole femur. With normal aging, the femoral neck asymmetrically and progressively loses bone tissue precisely where the cortex is already thinnest and is compressed in a fall. At the microscopic scale of the basic remodelling unit (BMU), increased numbers of actively remodelling BMUs associated with reduced mechanical loading in a typically inactive old age augments the numbers of mechanical flaws in the structure potentially capable of initiating cracking. Menopause and

over-deep osteoclastic resorption are associated with incomplete BMU refilling leading to excessive porosity, cortical thinning and dis-connection of trabeculae. In the femoral cortex, replacement of damaged bone or bone containing dead osteocytes is inefficient, impeding the homeostatic mechanisms that match strength to mechanical usage. In consequence the participation of healthy osteocytes in crack-impeding mechanisms is impaired. Observational studies demonstrate that protective crack deflection in the elderly is reduced. At the most microscopic levels attention now centres on the role of tissue aging, which may alter the relationship between mineral and matrix that optimises the inhibition of crack progression, on the role of osteocyte aging and death that impedes tissue maintenance and repair and on one newly revived and one quite new topic: the potentially key role of citrate in binding crystal to matrix and regulating crystal growth; and the potential role of repeated moderate trauma to cause de-lamination of cortical bone.

W6.3
Preclinical Methods for Assessing Bone Quality

Eleftherios P. Paschalis
Austria

Bone is a composite material consisting of mineral, organic matrix, and water. Its resistance to fracture is determined by its amount and its quality, the latter an umbrella term encompassing its structural and material properties. Metabolic bone diseases manifesting fragility fractures (such as osteoporosis) are routinely diagnosed based on bone mineral density (BMD) and biochemical markers measurements. Although clinically useful, it is nowadays well accepted that these measures do not fully account for fracture incidence. The emergence of bone quality led to the development of a plethora of analytical techniques (analysing bone tissue at both the macro and the micro scale) that provide information on all 3 components of bone (mineral, organic matrix, and water). Nevertheless, it is unlikely that a single outcome is responsible for fracture occurrence, as fracture is believed to be the culmination of a series of events. As a result, a combinatorial approach is preferred when possible. In this presentation, the most widely used techniques for preclinical determination of bone quality will be presented. For each, what is actually measured and what is inferred will be discussed, and emphasis will be placed on potential pitfalls. Finally, the example of Idiopathic Osteoporosis will be presented so as to highlight the importance of bone quality determination in the understanding of the pathophysiology of fragility fractures.

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CABS PARALLEL PROGRAMME

The 4th Joint Meeting of ECTS and IBMS
Rotterdam, The Netherlands
25–28 April 2015

CABS 1.1

Coupling of Angiogenesis and Osteogenesis by a Specific Vessel Subtype in Bone

Anjali P. Kusumbe, Saravana K. Ramasamy, and Ralf H. Adams
Muenster, Germany

The mammalian skeletal system harbours a hierarchical system of mesenchymal stem cells, osteoprogenitors and osteoblasts sustaining lifelong bone formation. Osteogenesis is indispensable for the homeostatic renewal of bone as well as regenerative fracture healing, but these processes frequently decline in ageing organisms leading to loss of bone mass and increased fracture incidence. There is evidence indicating that the growth of blood vessels in bone and osteogenesis are coupled, but relatively little is known about the underlying cellular and molecular mechanisms. Here we identify a new capillary subtype in the murine skeletal system with distinct morphological, molecular and functional properties. These vessels are found in specific locations, mediate growth of the bone vasculature, generate distinct metabolic and molecular microenvironments, maintain perivascular osteoprogenitors, and couple angiogenesis to osteogenesis. The abundance of these vessels and associated osteoprogenitors was strongly reduced in bone from aged animals, which was pharmacologically reversible to restore bone mass.

CABS 1.2

Molecular Stromal Signatures in the Supportive Bone Microenvironment of Breast and Prostate Cancer

Janine Hensel
Bern, Switzerland

Cancer cell growth is highly dependent on a growth permissive microenvironment (stroma). Prostate and mammary cancer (PCa and MCa) cells preferentially metastasize to bone, where they induce either an osteoblastic or osteolytic response. These opposite stromal responses suggest that different cancers adopt distinct strategies to hijack the bone marrow/bone stroma for their growth support. However, the molecular cues underlying these divergent responses are largely elusive. We exploited the sufficient divergence between human and mouse RNA sequences to dissect the stroma (mouse) from the cancer cell (human) transcriptome in bone metastasis xenograft models of human osteoinductive PCa cells (VCaP and C4-2B) and of pro-osteolytic PCa and MCa cells (PC-3 and MDA-MB-231, respectively). A robust induction of genes involved in osteogenesis and angiogenesis dominates the stroma response in osteoblastic bone metastasis. This translates in an amplification of hematopoietic and, remarkably, prostate epithelial stem cell niche components that may function as a self-reinforcing bone metastatic niche. The induction of this combinatorial stem cell niche is a novel mechanism that may also explain cancer cell osteotropism and the local interference with hematopoiesis (myelophthisis). Angiogenesis and skeletogenesis are the predominant biological processes also in the stroma response to osteolytic bone metastasis. However, this stroma transcriptome differs substantially from that of osteoblastic lesions and reveals not only activation of pro-osteoclastogenic signals, but also interference with pro-osteoblastogenic factors. Thus, the osteolytic lesions seem to be not only the result of exaggerated bone resorption, but also of inhibition of bone formation. Importantly, the stem cell niche type and molecular components amplified are markedly different between osteoblastic and osteolytic lesions. This suggests different growth support requirements between osteinductive and pro-osteolytic cancer cells and, thus, the need for a differential therapeutic targeting aiming at interfering with tumour growth in osteoblastic and osteolytic lesions.

CABS1.3

Abstract not available

CABS1.4

The Bone Microenvironment and Myeloma

Nicola Guiliani
Parma, Italy

Multiple myeloma (MM) is a plasma cell malignancy characterized by a tight relationship with the bone microenvironment cells. MM cells induce a significant alteration of the bone remodelling process due to the increase of osteoclast formation and activation and to the suppression of osteoblast differentiation leading to the impairment of bone formation and the development of osteolytic lesions. Recently an increase of osteocyte death has been also demonstrated in MM patients suggesting a potential role of these cells in the alterations of bone remodelling in MM. Interestingly, the increased osteoclastogenesis and the impaired bone formation in turn support myeloma cell proliferation and survival in vitro and promote tumoral progression in vivo. In addition it has been reported that quiescent myeloma cells with stem cell features may reside in the hypoxic osteoblastic niche for protection from apoptotic stimuli and are involved in the drug resistance and the relapse of the disease. Several studies have investigated the mechanisms involved in the relationship between myeloma cells and

bone microenvironment cells. MM cells are able to stimulate the osteoclastogenesis and to suppress osteoblast formation and viability either through the release of soluble factors or the cell-to-cell contact. Recently the potential role of *miRNAs* has been also suggested in the mechanisms involved in the suppression of osteoblast differentiation in MM. The identification of potential pathways involved in the relationship between bone and myeloma cells has leaded to identify new therapeutic targets including RANKL, Activin-A, Sclerostin, HIF-1α, and Wnt signaling pathways. Several new drugs have been developed and are under investigation for their future use in MM patients.

CABS 2.1

Radium-223 and Skeletal Metastases with Emphasis on the Osteoblastic Stroma

Oyvind Bruland
Oslo, Norway

Skeletal metastases are present in the vast majority of patients with castrate resistant prostate cancer (CRPC). The pronounced bone-tropism of disseminated tumour cells and the sclerotic phenotype of the metastases provide the basis for therapeutic use of bone-seeking radiopharmaceuticals (1). This lecture aims to discuss the mechanisms of action by which the bone-targeting alpha-emitter ^{223}Ra prolongs overall survival in patients with skeletal metastases form CRPC (2,3). Radium-223 in a chloride formulation deposits high-LET radiation within targeted sites following i.v injection (4). In a randomized Phase-2 trial, where 4 monthly injections of ^{223}Ra were given after external beam radiotherapy to the dominating painful site, a profound reduction of the alkaline phosphatase (ALP) bone-isoenzyme was observed (5). Also a significant decline in prostate specific antigen (PSA) was demonstrated with a survival benefit (5). This paved the way for the pivotal phase 3 ALSYMPCA study (2) that included 921 CRPC pts with bone mets (^{223}Ra, n=614; placebo, n=307). Here ^{223}Ra significantly improved overall survival vs placebo (median 14.0 vs 11.2 mo; HR=0.695; *P*=0.002) and was well tolerated. Radium-223 was in 2013 approved by EMA and FDA as the first-in-class alpha-emitter with a potent, targeted, anti-tumour effect on bone metastases and a favourable safety profile. In a post-hoc analysis (3), ^{223}Ra prolonged time to first symptomatic skeletal event versus placebo (median: 15·6 months *vs* 9·8 months, respectively; HR=0·66; 95% CI 0·52–0·83; p<0·001), and reduced both the risk of external beam radiation therapy for bone pain (HR=0·67; 95% CI 0·53–0·85) and spinal cord compression (HR=0·52; 95% CI 0·29–0·93). At the outset of the ^{223}Ra- development, the prevailing paradigm was that only the reactive zone surrounding the growing skeletal metastases (interphase between bone & cancer) expressed the osteoblastic stroma. In CRPC, however, there is a mesh of reactive stroma avid for ^{223}Ra entwined between cords of carcinoma cells (1) with ALP-expression by the CRPC cells per se; due to osteomimicry and epithelial-mesenchymal transition. This may impact on further clinical development of ^{223}Ra.

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CABS 2.2

Preclinical Developments; Radium-223 in Osteolytic Bone Metastases and Osteosarcoma?

Michel Wissing
Leiden, The Netherlands

In 2013, the United States Food and Drug Administration approved Radium-223 for the treatment of prostate cancer patients with bone metastases, based on the results of the ALSYMPCA trial. In doing so, Radium-223 became the first approved anticancer agent that extended overall survival in cancer patients not by directly targeting the tumor, but by targeting its (skeletal) metastases. Radium-223 may effectively target bone metastases in other tumors as well, such as breast and lung cancer. Furthermore, considering its mechanism of action, it is likely that Radium-223 will have antitumor activity in primary bone cancers such as osteosarcomas too. In this lecture, results of studies with Radium-223 in tumors other than prostate cancer will be discussed.

CABS 3.1

Mechanisms of Cancer Invasion and Metastasis: Potential Therapeutic Implications

Peter Friedl
Nijmegen, The Netherlands & Houston, USA

Bone The tumor microenvironment contributes to cancer invasion, growth and survival and thereby impacts tumor responses to therapy. We here developed an intravital infrared multiphoton imaging model for the multi-parameter visualization of collective cancer cell invasion, guidance by the tumor stroma, and short- and long-term resistance to experimental anti-cancer therapy. Using orthotopic fibrosarcoma and melanoma xenografts, we identify deep invasive growth driven by proliferation concurrent with collective invasion as main local invasion route, which further mediated resistance to high-dose hypofractionated radiation therapy (cumulative dose 20 to 40 Gy). This invasion-associated radioresistance niche comprised several hundreds of cells in close proximity to stromal structures, including collagen, basement vascular and myofibre membranes, and was able to re-establish tumor growth and relapse, thus escaping other imaging modalities but in vivo microscopy. Using simultaneous inhibition of β1

and β3 integrins by RNA interference or combined anti-β1/αV integrin antibody treatment, however, proliferation arrest, anoikis induction was achieved, ablating both tumor lesion and the resistance niche. Thus, the invasion niche represents a microenvironmentally privileged survival niche which provides integrin-dependent therapy resistance. To establish an model amenable to intravital multiphoton microscopy of bone metastases of prostate cancer (PCa), we implanted engineered humanized neobone into the mouse dermis. After *in vivo* implantation, TEBC maturation was monitored by μCT, MPM and histological analysis over time to generate a miniaturized-neobone with defined cortical thickness (50-60 mm) surrounding histologically mature murine bone marrow. PCa (PC3) lesions, after implantation into the bone cavity, were longitudinally monitored for growth, niche development and step-wise osteolysis, using multi-parameter recording of collagen/bone matrix, bone surface, blood vessels, stromal phagocytes and steoclasts, and PC3 cells. By combining innovative tissue engineering with optical windows, state-of-the-art fluorescence reporter technology and intravital MPM, this model will provide mechanistic and applied insight into the therapy response of bone metastases.

CABS 3.2

Abstract not available

CABS 3.3

CTCs – Who Cares? It is all about the DTCs!

Ingunn Holen
Sheffield, UK

We have limited understanding of the relationship between CTCs and DTCs and to study these populations in clinical samples remains technologically challenging. CTCs are heterogeneous and the majority may not ultimately form metastases. So what will a detailed analysis of their genetic makeup really tell us? In addition, collection of DTCs most commonly involves bone marrow aspirates that potentially do not capture DTCs embedded in specific bone niches. Few studies have included collection and comparison of CTCs and DTCs from the same patient. The use of *in vivo* model systems combined with recent technological advances in cell labelling and imaging has provided new insights into the early stages of tumour cell dissemination to the skeleton. In particular, the role of specific microenvironments or niches, as well as how DTCs residing within these niches are affected by environmental signals and therapeutic targeting, is emerging. But to what extent can this information be translated to human disease? This lecture will provide a summary of the current understanding of CTCs and DTCs in development of metastatic disease and show the latest pre-clinical data from studies of DTCs in breast/prostate cancer. The role of CTCs and DTCs will be discussed; should CTCs be used as a measure of disease burden whereas DTCs should be the main therapeutic target? How useful is CTC gene expression data for patient outcome? Do CTC have to become DTCs in order to pose a risk?

CABS 4.1

Long Non-coding RNAs in Prostate Cancer Progression

Guido Jenster
Rotterdam,The Netherlands

Current prostate cancer (PCa) biomarkers such as PSA are not optimal in distinguishing cancer from benign prostate diseases and predicting disease outcome. To discover additional biomarkers, we investigated PCa-specific expression of novel unannotated transcripts. Using Affymetrix Human Exon Arrays and RNA sequencing data, we identified 334 candidates referred to as EMC PCa-associated transcripts (EPCATs). These transcripts are uniquely expressed in subsets of PCa and not or barely expressed in normal prostate and other tissues. To validate their unique expression pattern, 15 EPCATs were validated by RT-PCR in cell lines and patient samples. Combined into a diagnostic panel, 11 EPCATs classified 80% of PCa samples correctly, while maintaining 100% specificity. High specificity was confirmed by *in situ* hybridization for EPCAT4R966 and EPCAT2F176 (SChLAP1) on extensive tissue microarrays. Besides being diagnostic, EPCAT2F176 and EPCAT4R966 showed significant association with pT-stage and were present in cancer precursor PIN lesions. We also found EPCAT2F176 and EPCAT2R709 to be associated with development of metastases and PCa-related death, and EPCAT2F176 to be enriched in lymph node metastases. Functional significance of expression of 9 EPCATs was investigated by siRNA transfection, revealing that knockdown of 5 different EPCATs impaired growth of LNCaP and 22RV1 PCa cells. Two EPCATs inhibited the migration of PC3 cells in a Boyden chamber assay. The EPCATs investigated so far do not exhibit a protein coding potential and are classified as long noncoding RNAs (lncRNAs). Ours and many other studies have now shown that our transcriptome consists of a limited number of coding RNAs (~22,000) and a huge variety of small and long noncoding transcripts. Surprisingly, many of these transcripts are uniquely expressed in one type of tissue or cancer. The observation that some of these lncRNAs are functionally relevant indicates that they do not just reflect random disease-related changes, but rather another layer of cellular regulatory complexity. Although the underlying transcriptional regulation is not fully understood, the novel PCa-associated transcripts are new diagnostic and prognostic markers with functional relevance to prostate cancer growth.

CABS 4.2

Abstract not available

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HOT TOPICS

The 4th Joint Meeting of ECTS and IBMS
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HT1

High Serum Levels and Liver Expression of Sclerostin in Patients with Primary Biliary Cirrhosis. Association with Markers of Bone Remodelling and Severity of Cholangitis

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Sclerostin is involved in the regulation of osteoblastogenesis and little is known about its role in the development of bone disease in primary biliary cirrhosis (PBC), characterised by low bone formation. Therefore, we have assessed the circulating levels and the liver expression of sclerostin in this cholestatic disease. Serum sclerostin levels were measured in 83 women with PBC (mean age: 60±12 years) and 101 control women. Lumbar and femoral BMD as well as parameters of mineral metabolism and bone remodeling were measured. Moreover, sclerostin gene expression in the liver was assessed in samples of liver tissue taken by biopsy in 11 PBC patients and 5 healthy controls by real time PCR, and presence and distribution of sclerostin was evaluated in liver slices from 11 patients by immunohistochemistry. The presence and severity of histologic lesions were assessed semiquantitatively in the same liver samples. 77% of patients had low BMD (22% osteoporosis and 55% osteopenia). PBC patients had higher sclerostin levels than controls (76.7±38.6 vs. 32.5±14.7 pmol/L, $p<0.001$). Serum sclerostin correlated inversely with markers of bone formation and resorption. Sclerostin mRNA in the liver was overexpressed as compared with control samples (2.7±0.3 fold vs healthy liver). Sclerostin was detected by immunohistochemistry in 7 of the 11 liver samples and mainly located in the bile ducts. Sclerostin was associated with the severity of cholangitis ($p=0.02$) and indirectly with the degree of lobular inflammation ($p=0.03$). Sclerostin mRNA expression was higher in samples positive by immunohistochemistry (2.9±0.4 vs 2.5±0.3, p:n.s.), and particularly in those with lobular granuloma (3.6±0.6 vs 2.4±0.2, $p=0.02$). The increased expression of sclerostin in the liver and the association with histologic cholangitis may explain the high serum levels of this protein in patients with PBC, thus suggesting that sclerostin influences the decreased bone formation in this cholestatic disease.
Disclosure: The authors declared no competing interests.

HT2

Osteocyte-Specific Ablation of Pparγ Results in Sost Down-Regulation and Increased Periosteal Bone Formation but Decreased Bone Turnover in Mice

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While heterozygous Pparγ-deficient mice exhibited high bone mass with increased osteoblastogenesis from bone marrow progenitors, the role of Pparγ specifically in late osteoblast/osteocyte (ocy) is not yet properly understood. We crossed Pparγ-loxP with Dmp1-cre mice to generate Ocy-Pparγ$^{-/-}$ and -Pparγ$^{+/+}$ male mice, which developed normally until 3 months of age, at which time they were analyzed in details. Tissue and cell specificity of Pparγ deletion were confirmed respectively by western blot and immunostaining. Total body lean & fat mass as well as handgrip strength were comparable in Ocy-Pparγ$^{-/-}$ and -Pparγ$^{+/+}$ mice. Femoral BMD was significantly higher in Ocy-Pparγ$^{-/-}$ (78.6±1.3 vs 73.4±2.1 mg/cm^2 in Pparγ$^{+/+}$, $p<0.001$). Trabecular and cortical microarchitecture respectively evaluated at the distal and midshaft femur was improved in Ocy-Pparγ$^{-/-}$: BV/TV +28.6%, TbTh +12.5%, CtTV +9.8%, CtBV +12.0% and CtTh +4.5% compared to Pparγ$^{+/+}$ (all $p<0.05$). Periosteal bone forming rate was higher in Ocy-Pparγ$^{-/-}$ (+66.5% vs Pparγ$^{+/+}$, $p<0.05$), whereas no significant differences were observed at endocortical surfaces. In contrast CTx was decreased in Ocy-Pparγ$^{-/-}$ (8.6±0.6 vs 12.9±0.5 ng/ml in Pparγ$^{+/+}$, $p<0.001$). Gene expression analyses in the osteocytic fraction of cells extracted from the femur showed lower *Sost* mRNA levels, 50% lower in Ocy-Pparγ$^{-/-}$ compared with Pparγ$^{+/+}$, whereas *Opg* or *RankL* mRNA levels were comparable. However, in the osteoblastic fraction, both *Runx2* and *Opg* levels were significantly higher in Ocy-Pparγ$^{-/-}$, respectively +19.2% and +197.6% vs Pparγ$^{+/+}$, both $p<0.01$. In conclusions, Ocy-specific ablation of Pparγ down-regulates *Sost* and upregulates *Opg* expression, resulting in increased periosteal bone formation but lower bone turnover and high bone mass. These observations suggest a role of Pparγ in osteocytes on the control of bone modelling and remodelling by these cells *in vivo*.
Disclosure: The authors declared no competing interests.

HT3

Intensive Bisphosphonate Therapy Increases the Risk of Fracture and Requirement for Orthopaedic Surgery in Paget's Disease: the PRISM-EZ Study

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The optimal management strategy for Paget's disease of bone (PDB) remains to be established although recent clinical guidelines have suggested that bisphosphonates should be given to maintain alkaline phosphatase (ALP) concentrations within the mid reference range. We report upon the long term outcome of treatment in PDB within the PRISM-extension with Zoledronic acid study (PRISM-EZ) in which 502 PDB patients, who all participated in the PRISM study, were followed for an additional 3 years. There were two treatment groups; symptomatic therapy (n=232) where bisphosphonates were only given if symptoms were present and intensive therapy (n=270) where bisphosphonates were given with the aim of maintaining ALP within the reference range. Zoledronic acid was the bisphosphonate of choice in the intensive group. Mean concentrations of ALP were in the mid-reference range in the intensive group throughout the study (mean ± SD normalised ALP = 0.71±0.30) but at the upper end of the reference range in the symptomatic group (1.01±0.81; p<0.001 between groups). There was no significant difference between treatment groups in bone pain and quality of life scores assessed by the SF36 questionnaire. Thirty-eight patients suffered a fracture (23 in the intensive and 15 in the symptomatic treatment groups, respectively). Six out of the above 38 patients had fracture of bone affected by PDB. Sixteen patients required orthopaedic surgery. Patients randomised to receive intensive bisphosphonate therapy were more likely to experience a fracture or undergo orthopaedic surgery than those in the symptomatic arm (Hazard ratio 1.94, 95% CI 1.07-3.51, p=0.029) and this remained significant after correction for baseline characteristics (HR 1.85, 95% CI 1.02-3.38, p=0.044]. We conclude that intensive bisphosphonate therapy confers no benefit in patients with PDB and on the contrary may be harmful. Treatment should be directed at patient symptoms rather than keeping ALP concentrations within the reference range.

Disclosure: Stuart H Ralston acts as a consultant for Novartis and Merck on behalf of his institution, the University of Edinburgh. William D Fraser acts as a consultant for Procter & Gamble, MSD, Novartis, Sanofi Aventis, Nycomed and Roche. The remaining authors have no interests to declare. The PRISM-EZ study was supported by a grant from Arthritis Research UK.

HT4

Inhibition of Vascular Calcification by Extracellular Nucleotides, P2 Receptors and NPP1

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Vascular calcification (VC) shares some similarities to skeletal mineralisation, and involves hydroxyapatite deposition in arteries and cardiac muscle. Whilst VC has severe clinical consequences, the cellular mechanisms responsible are not fully elucidated. ATP and UTP (≥1μM) inhibit bone mineralisation via P2 receptor-dependent (P2R) and independent mechanisms. The latter involves the hydrolysis of extracellular nucleotides by NPP1 to produce pyrophosphate (PP_i), a key mineralisation inhibitor. This study investigated whether extracellular nucleotides also regulate VC. Vascular smooth muscle cells (VSMCs) were cultured in calcifying medium containing 2.5 mM phosphate for 14 days. We found that VSMCs express multiple P2Rs and expression was up-regulated in calcifying conditions. The key source of extracellular ATP is controlled release from cells: removal of endogenous ATP by apyrase (an ecto-nucleotidase which hydrolyses ATP) resulted in a 45% increase in VSMC calcification. Culture with exogenous ATP and UTP (≥1 μM) decreased VSMC calcification by ≤80% and 90%, respectively (p<0.001). The selective agonists, 2-thioUTP and MRS2768, also inhibited VSMC calcification by <70% (p<0.001) suggesting a role for the $P2Y_2R$ in mediating these effects, Furthermore, the level of calcification is increased 2-fold in VSMCs from $P2Y_2R$ knockout mice. VC is associated with increased VSMC apoptosis and a transdifferentiation of VSMCs towards the osteogenic lineage. We observed that ATP/UTP increased VSMC number and decreased the expression of genes associated with osteoblast differentiation (e.g. Runx2, osterix, Ocn). CTP and GTP (≥10μM), which are not P2R agonists but are hydrolysed by NPP1 to produce PP_i, blocked VSMC calcification by ≤70%. Furthermore, in NPP1 knockout VSMCs, the inhibitory actions of ATP and UTP were 10-fold less potent. These results indicate the P2R-independent mechanisms (involving PP_i) contribute significantly to the inhibitory actions of extracellular nucleotides on VC. Taken together, our data suggest an important role for extracellular nucleotides, the $P2Y_2R$ and NPP1 in the regulation of VC.

Disclosure: The authors declared no competing interests. This work was supported by Arthritis Research UK (#19205).

HT5

Abstract not available

HT6

High Periostin Levels in Cortical Bone of Cathepsin K Knock-Out Mice are Responsible for Increased Periosteal Bone Formation and Bone Mass

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Cathepsin K (CatK) inhibition in preclinical models results not only in lower bone resorption but also in higher bone formation (BF) on both remodeling and modeling surfaces. The mechanisms for greater BF at modeling surfaces remain unexplained. *In vitro* data suggest that periostin is proteolytically degraded by CatK. Periostin (Postn) is a matricellular protein expressed in the periosteum and osteocytes that mediates β-catenin signalling and skeletal response to loading. We hypothesised that higher BF in *Ctsk*$^{-/-}$ mice might be related to increased periostin expression. For this purpose, BMD, microstructure and histomorphometry were evaluated in the progeny of a *Postn*$^{+/-}$ X *Ctsk*$^{+/-}$ mouse cross. Postn immunostaining was more intense in *Ctsk*$^{-/-}$ osteocytes and periosteum surfaces vs WT. *Postn*$^{+/+}$;*Ctsk*$^{-/-}$ have higher BMD, BV/TV, CtBV, CtTh, whereas *Postn*$^{-/-}$;*Ctsk*$^{+/+}$ have lower femoral BMD, CtBV, and CtTh compared to WT. *Postn*$^{+/+}$;*Ctsk*$^{-/-}$ have higher Ps-MAR and –BFR (+58% and + 137% vs WT, $p<0.05$), as well as Ec-MAR (+74% vs WT, $p<0.05$) but lower Ec-MPm/BPm (-40% vs WT, $p<0.05$), indicating that BF is increased on both cortical envelopes and independently of remodeling surfaces. Removing both Postn alleles in *Ctsk*$^{-/-}$ mice, i.e. *Postn*$^{-/-}$;*Ctsk*$^{-/-}$, prevented the increase in CtBV and Ps-BFR, but had no effect on Ec-MPm/BPm which remained low (-51% vs WT, $p<0.05$), indicating that Postn mediates cortical bone formation but has no influence on CatK dependent bone remodeling. In contrast, trabecular microarchitecture at the distal femur and vertebrae was not affected by Postn deletion in *Ctsk*$^{-/-}$ mice, indicating that Postn-dependent bone formation in these mice occurs specifically at cortical surfaces. In conclusions, periostin expression is increased in *Cstk*$^{-/-}$ mice and is responsible for the increased modelling-based bone formation observed on cortical bone in the absence of cathepsin K.

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ORAL COMMUNICATIONS

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OC1.1
Wnt16 Promotes Osteoblastogenesis and is Negatively Regulated by Glucocorticoids *In Vitro* and *In Vivo*

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Glucocorticoids (GCs) are effective drugs to treat inflammatory diseases, but exert detrimental effects on bone when used over longer periods of time. One of the main mechanisms of GC-induced bone loss is the suppression of osteoblast activity. Osteoblast-derived Wnt16 has recently been shown to determine cortical bone mass by regulating osteoclast function. However, its role in osteoblastogenesis and its regulation by GCs remain unknown. Here, we assessed the role of Wnt16 during osteoblast differentiation and tested whether GCs regulate Wnt16 expression. Wnt16 was highly expressed in primary murine bone marrow stromal cells (BMSCs), promoted osteoblastogenesis and activated canonical Wnt signalling in MC3T3-E1 cells. GC treatment using dexamethasone (DEX) decreased Wnt16 mRNA expression levels by 50% *ex vivo* in BMSCs. Wnt16 suppression was dose-and time-dependent, reaching a maximum after 48 h at a concentration of 1 μM. Consistently, treatment of mice with GC-containing slow-release pellets for two weeks reduced vertebral bone mineral density by 13% and Wnt16 mRNA levels by 35% in bone tissue. The suppression of Wnt16 by GCs was strictly GC receptor (GR)-dependent. Co-treatment of BMSCs with DEX and the GR antagonist RU-486 completely abrogated the GC-mediated suppression of Wnt16. Likewise, DEX failed to suppress Wnt16 expression in BMSCs derived from GR knockout mice. Additionally, Wnt16 mRNA levels were unaltered after GC treatment in bone tissue of GR^{dim} mice, which lack the ability of GR dimerisation and therefore binding of the GR to DNA, suggesting that GCs suppress Wnt16 via direct DNA-binding mechanisms. In line with this, DEX treatment reduced Wnt16 promoter activity in MC3T3-E1 cells. Thus, this study underlines the pro-osteogenic effect of Wnt16 and identifies Wnt16 as a novel GC target. As the suppression of Wnt16 could define a mechanism of reduced osteoblast activity, Wnt16 may represent a novel target for therapeutic intervention in GC-induced bone loss.
Disclosure: The authors declared no competing interests.

OC1.2
Osteoblast N-Cadherin Restrains Wnt/β-Catenin Signalling and the Osteo-Anabolic Effect of Dkk1 Inhibition

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We and others have shown that N-cadherin (Ncad) physically interacts with low density lipoprotein receptor-related protein-5 or 6 (Lrp5/6) and Axin, resulting in negative regulation of canonical Wnt/β-catenin signalling in osteoblasts. We tested whether removal of the N-cadherin gene (*Cdh2*) alters bone mass accrual in response to Lrp5/6 signalling. We administered a Dickkopf-1 (Dkk1) neutralizing antibody (αDkk1) to activate Lrp5/6 in mice with conditional *Cdh2* ablation driven by the 2.3 *Col1A1* promoter (Col1-cKO). At the dose of 5 mg/kg body weight i.p., 3 times/week for 4 weeks, αDkk1 was ineffective in WT mice, but produced a 2-fold increase of BV/TV (0.496±0.085 $p<0.01$ vs. baseline) in *Col-Cdh2* cKO mice. A higher dose (20 mg/kg) was equally effective in both genotypes. At the molecular level, a single dose of αDkk1 produced accelerated accumulation of β-catenin in bone of Col1-cKO relative to WT mice, indicating direct Wnt/β-catenin signalling activation in bone by αDkk1 and enhanced responsiveness in the absence of Ncad. To corroborate this finding, we introduced one Dkk1-resistant $Lrp5^{A214V}$ allele associated with high bone mass (HBM), in conditionally *Cdh2* ablated mice driven by *Osx1-Cre* (Osx1-cKO). Although bone mass (by μCT) was lower in Osx1-cKO than in WT, the compound $Lrp5^{A214V}$;*Cdh2* cKO mutants were osteosclerotic and indistinguishable from $Lrp5^{A214V}$ HBM mice. Despite lower total β-catenin abundance in Osx1-cKO bone marrow stromal cells, steady state cytosolic β-catenin was not decreased. Upon Wnt3a stimulation, N-terminally un-phosphorylated β-catenin was more abundant in Osx1-cKO than in WT cells, suggesting higher levels of active β-catenin in the absence of N-cadherin in response to Wnt3a. In summary, mice lacking *Cdh2* in osteolineage cells are hyper-responsive to Wnt signaling activation and to its osteo-anabolic effect. These results provide *in vivo* proof of the concept that Ncad restrains anabolic Lrp5/6 signalling in bone forming cells.
Disclosure: The authors declared no competing interests.
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OC1.3

Mechanical Loading Reduces Inflammation-Induced Human Osteocyte-to-Osteoclast Signalling

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Multiple factors contribute to bone loss in inflammatory diseases such as rheumatoid arthritis (RA), but circulating inflammatory factors and immobilisation play a crucial role. Mechanical loading prevents bone loss in the general population, but the effects of mechanical loading in patients with RA are less clear. Therefore, we aimed to investigate whether mechanical stimuli can reverse the modulatory effects of circulatory inflammatory factors present in RA-serum on osteocyte-to-osteoclast signalling. We also investigated whether inflammatory factors present in RA-serum alter the response of osteocytes to mechanical stimuli. Human primary osteocytes from trabecular bone pieces were treated with 10% serum from active RA patients or healthy controls for 7 days. Then cells were subjected to 1 h mechanical loading by pulsating fluid flow (PFF; 0.7±0.7 Pa, 5 Hz) or static control culture, and medium NO and PGE2 concentrations were determined. Cells were post-incubated without PFF for 1h, and cytokine gene expression was quantified by qPCR. Osteoclast precursors were cultured for 21 days with PFF-conditioned medium (PFF-CM) or static-conditioned medium (stat-CM) collected after 1h post-incubation, and osteoclast formation was assessed. RA-serum did not affect IL-6, CYR61, COX2, MEPE, or SOST gene expression in osteocytes. However it enhanced the RANKL/OPG expression ratio by 3.4-fold, while PFF nullified this effect. PFF enhanced NO production in both control-serum and RA-serum–pretreated osteocytes, while PFF only enhanced PGE2 production in control-serum–pretreated osteocytes. Stat-CM from RA-serum–pretreated osteocytes enhanced osteoclastogenesis compared with stat-CM from control-serum–pretreated osteocytes, while PFF-CM from RA-serum–pretreated osteocytes nullified this stimulatory effect on osteoclastogenesis. PFF-CM from control-serum–pretreated osteocytes also inhibited osteoclastogenesis. In conclusion, RA-serum containing inflammatory factors did not alter the intrinsic capacity of osteocytes to sense mechanical stimuli, but induced osteocyte-to-osteoclast communication, while mechanical loading nullified this effect, suggesting that mechanical stimuli could contribute to the prevention of osteoporosis in RA.

Disclosure: The authors declared no competing interests.

OC1.4

RNA interfering Strategy to Cure Autosomal Dominant Osteopetrosis Type 2 (ADO2)

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Genetic autosomal dominant diseases are generally due to heterozygous missense mutations that could be eradicated by RNA interference. We hypothesised that this approach could cure ADO2 and tested this treatment in ADO2 mice carrying a G213R amino acid substitution in the CLC-7 protein, encoded by the Clcn7 gene. Using a systematic mutation-driven strategy, we designed and tested in-vitro various small interfering (si)RNAs against this mutation and found a Clcn7G213R-siRNA that silenced specifically the mutant transcript in transfected HEK293 cells (-85%,p=0.02), without affecting the WT gene and rescuing bone resorption in ADO2 osteoclasts (+2.6-fold, p=0.003). This siRNA was made "sticky" by 3'dAdT overhangs, conjugated with the delivery system 'in-vivo-JetPEI®' and injected i.p. in ADO2 mice. Time- and dose-dependent experiments evidenced 4 mg/Kg every 48 h to be the most effective treatment, decreasing the mutant mRNA in tibias (-80%, p=0.01). Two-weeks treatment of ADO2 mice, down-regulated Clcn7G213R mRNA expression in bone and other organs, increased the serum bone resorption marker CTX over the osteoclast marker TRAcP 5b (+1.8-fold, p=0.002), and decreased trabecular BV/TV (-19%, p=0.04) and Tb.N (-16%, p=0.05) vs scrambled-siRNA treated ADO2 mice. After 4 weeks, trabecular BV/TV (-21%, p=0.03) and trabecular variables (Tb.N -19%, Tb.Sp +1.2-fold, p<0.03) returned to WT level, with a full rescue of the bone phenotype. In the rescued ADO2 mice, serum CTX/TRAcP, osteoclast number and erosion surface/bone surface were normalised (+1.2-fold, -32%, +2.1-fold, p=0.03, p=0.01, p=0.02, respectively, vs. control ADO2 mice), while osteoblast and dynamic parameters were unremarkable. Treatment was well tolerated, with no adverse events, and with normalisation of liver aspartate aminotransferase. To the best of our knowledge, this is the first experimental curative treatment of ADO2, which rescued osteoclast function and returned the bone phenotype to normal by a systemic RNA interference strategy. The invention is protected by the patent application RM2014A000272, which could provide the means to develop this siRNA strategy for therapy in humans.

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OC1.5

Dominant Mouse Model with Uncleavable Type I Collagen C-propeptide Processing Site has Extremely Brittle Bones

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Classical osteogenesis imperfecta (OI) is caused by type I collagen mutations. Mutations in the C-propeptide cleavage site of both *COL1A1* and *COL1A2* cause high bone mass OI, characterised by bone hypermineralisation. To elucidate the role of type I procollagen C-propeptide processing in bone formation, we generated a mouse with a heterozygous C-propeptide cleavage site defect (high bone mass, HBM), substituting both COL1A1 cleavage site residues to prevent BMP1 cleavage. Western blots on long bone extracts revealed unprocessed pro- and pC-collagen and cleaved C-propeptide in HBM bone. At 2 months, male HBM mice are significantly smaller in weight (77%) and length (92%) and have shorter femurs (92%). All 2

month HBM mice have pelvic deformities; 40% have kyphosis. Femoral aBMD in HBM mice is decreased 25% ($p<0.001$), but vertebral BMD is normal. On µCT, HBM femora have thinner cortices with decreased cortical area. Four-point bending revealed significantly decreased HBM femoral stiffness, yield load, and ultimate load. HBM femora are also extremely brittle; post-yield displacement is only ~10% of WT (0.23 vs 0.03, $p<0.001$). Collagen from HBM calvarial osteoblasts had normal biochemistry with normal trimer incorporation, however, HBM osteoblasts deposited only about 50% of WT matrix. *Sost* transcripts in HBM femora are decreased ~40% and suggests C-propeptide processing may also influence cellular differentiation. Dermal fibril diameters were smaller and more homogeneous in HBM than WT, with loss of large fibrils. The HBM mouse phenotype is similar to that of the *Bmp1*$^{-/-}$/*Tll1*$^{-/-}$ mouse which also has small size, thin cortices, reduced maximum load and a dramatic decrease in post-yield displacement. The HBM mouse demonstrates that the essential elements of the broader enzyme deficiency are reproduced by a substrate defect in type I C-propeptide cleavage. These data show the importance of the type I procollagen C-propeptide to both collagenous and mineral properties of bone.

Disclosure: The authors declared no competing interests.

OC1.6

Combination Sclerostin Antibody and Zoledronic Acid Treatment Outperforms Either Treatment Alone in a Mouse Model of Osteogenesis Imperfecta

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Background: Osteogenesis Imperfecta (OI) is a genetic disorder featuring bone fragility and decreased bone mass. Bisphosphonates in children with OI reduce bone catabolism and rely on modelling to form new bone. An anabolic treatment, Anti-Sclerostin Antibody (Anti-SOST Ab), is being investigated in clinical trials. We hypothesised that combined treatment may produce superior outcomes.

Methods: Female Col1a2 G610C mice and their wild type (WT) littermates were treated from week 5 to week 9 of life to either saline (control), zoledronic acid (ZA) 0.025 mg/kg sc weekly, Anti-SOST Ab given 50 mg/kg IV weekly (Anti-SOST), or a combination of both (ZA Anti-SOST). Outcomes included weekly DEXA for areal bone mineral density (BMD) (GE Lunar PIXImus WI, USA), µCT (SkyScan 1174 Kontich, Belgium), mechanical testing of tibiae in 4 point bending (Instron 5944, Massachusetts, USA). Data were analysed with one-way ANOVA (SPSS v11).

Results: Increases in tibial BMD were seen over time in all groups. Anti-SOST treatment alone had no effect on tibial BMD, while ZA (16%) and ZA Anti-SOST (27%) treatments produced significant increases from weeks 1-4 ($P<0.05$). µCT analysis showed increases in Tissue Mineral Density and Cortical Thickness for combined treatment over respective controls. Tibial 4-point bending showed only combined ZA Anti-SOST yielded a significant increase in strength and energy to failure in OI mice, restoring bone strength to the values of untreated WT mice. In the spine, all treatments increased compression strength over control, Anti-SOST 30%, ZA 43% and ZA Anti-SOST 91% ($P<0.05$).

Conclusion: Anti-SOST Ab alone had effects on trabecular but not cortical sites in this study in Col1a2 G610C mice. Roschger *et al.* reported minimal effect in the Col1a1(Jrt)/+ mouse model treated with Anti SOST Ab, whereas large effects were noted with just 2 weeks treatment in 8 week-old Brtl/+ mice, leading to increase in bone size and strength. A combination of zoledronic acid and anti-sclerostin antibody is superior over either treatment alone in the Col1a2 G610C model of OI. Further studies are required in alternate mouse models of OI to confirm efficacy across different models, and thus to predict possible efficacy across the heterogeneous population of OI patients.

Disclosure: Little DG Research Contracts Novartis Pharma; Kneissel M, Kramer I Novartis Pharma employees; Anti-SOST Antibody supplied by Novartis Pharma.

OC2.1

Melatonin Improves Bone Mineral Density (BMD) at the Femoral Neck in Post-Menopausal Women with Osteopenia: a Randomised Controlled Trial

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Background: Melatonin is known for its regulation of circadian rhythm, however, over recent years, studies have shown that melatonin also has a positive effect on bone. With age, the melatonin levels decrease leading to further imbalanced bone remodelling. We aimed to investigate whether treatment with melatonin may improve bone parameters.

Method: In a double-blind placebo-controlled investigator initiated study, we randomised 81 healthy post-menopausal women with osteopenia to one-year of treatment with melatonin in a nightly dose of 1 mg (N=20), or 3 mg (N=20), or similar placebo (N=41). At baseline and after 12 months of treatment, DXA measurements of body composition, and BMD at the spine and hip were collected. Biochemical markers of calcium homeostasis were measured throughout the trial.

Results: Mean age was 63 (range 56-73) years. Compared with placebo, BMD at the femoral neck increased by 1.4% (95%CI:-2.7;-0.0, $p<0.05$) in response to melatonin. A dose-response relationship was present ($p<0.01$) as BMD at the femoral neck increased by 2.3% (95%CI:0.7;4.0, $p<0.01$) in the high dose (3mg/d) melatonin group compared with placebo. Compared with 1 mg/d of melatonin, BMD in the 3 mg/d group increased by 1.9% (95%CI:0.0;3.7, $p<0.05$). Treatment did not affect BMD at other skeletal sites or levels of bone turnover markers, however, there was a significant decrease in 24 h urinary calcium in the melatonin group (-3.7%, IQR:-2.9;57.0) compared with placebo (8.5%, IQR:-11.5;19.4, $p=0.02$). Moreover, compared with placebo, melatonin decreased fat mass significantly by 6.8% (95%CI: 1.3;12.3, $p=0.02$), while lean body mass increased by 2.2% (95%CI:-4.8;0.3,$p=0.08$).

Conclusion: One year of treatment with melatonin improved BMD dose-dependently at femoral neck and showed beneficial effects on body composition in terms of a reduced fat mass and borderline increased lean tissue. Further studies are needed to assess mechanisms of action and whether nighttime melatonin may protect against fractures.

Disclosure: The authors declared no competing interests.

OC2.2
Spontaneous Femoral Varization as a Risk Factor for Atypical Femoral Fractures

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Background: Several reports have linked bisphosphonates (BPs) with atypical femoral fractures (AFFs), but there is still debate regarding the real influence of these drugs on the development of such fractures. We speculated that AFFs could be related to lower limb geometry, specifically to spontaneous femoral varization, which would result in increased stress on the femoral cortices.
Methods: In order to test this hypothesis, we conducted a case-control study examining the geometric characteristics of the femur in patients who had suffered an AFF during treatment with BPs and in control patients taking BPs for a long time and not experiencing AFFs. A standing X-ray of the lower extremities was obtained. The following parameters were measured: curvature of the femur, distance from the femur to the load line, femoro-tibial angle and load angle. Eight women on BPs suffered 11 AFF (8 complete fractures and 3 incomplete fractures). Three patients had AFFs of both femora, the control group included 21 women with postmenopausal osteoporosis.
Results: The geometric features of patients with AFFs were very different from those of the control group, with a marked tendency to increased curvature of the femur in the patient's group (Table 1).
Conclusion: Our results suggest that patients with disturbed lower limb geometry are at higher risk of AFFs. Therefore, it may be worthwhile to obtain a standing X-ray of the lower legs in patients on long-term BPs in order to identify those individuals more susceptible to AFF.
Disclosure: The authors declared no competing interests.

Table 1 [OC2.2]: Geometric features

	CASES		CONTROLS		p
curvature of the femur (grade)	9	2-20	–4.4	(–10)-5	0.00004
distance from the femur to the load line (mm)	37.9	14-53	6.3	0-17	0.00004
femoro-tibial angle (grades)	4	1-9	–3.6	(–8)-17	0.0003
load angle (grades)	7.4	5-12	–7.6	(–11)-9	0.0002

OC2.3
Osteoprotegerin Autoantibodies are Independently Associated with Reduced Bone Density in Coeliac Disease

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Background: Autoantibodies neutralising the effect of the bone regulatory cytokine osteoprotegerin (OPG) have been described in a patient with severe osteoporosis and coeliac disease. This study aimed to determine the prevalence of autoantibodies to OPG in patients with coeliac disease, and correlate their presence with bone mineral density.
Methods: A direct enzyme linked immunosorbent assay using recombinant OPG as a capture antigen was developed and used to screen serum from 282 patients with coeliac disease for autoantibodies to OPG. Bone mineral density data was available in 254 patients. A threshold for the presence of OPG antibody was defined as the mean plus three standard deviations of values obtained from 102 healthy controls.
Results: OPG autoantibodies were found in 14/282 (5%) patients with coeliac disease. Bone mineral density results are summarised in table 1. The presence of OPG antibodies was associated with lower spine bone mineral density T and Z-scores on both univariate analysis, and multivariate analysis including age, sex, height and weight as covariates ($p<0.05$). This association was also seen when analysing the titre of OPG antibody as a continuous trait. A non-significant reduction in mean bone mineral density hip scores was seen in patients with OPG antibodies. (See table 1.)
Conclusion: Raised levels of OPG autoantibodies are found in 5% of patients with coeliac disease and are independently associated with reduced spine bone mineral density. Further work is required to establish the clinical utility of testing for OPG antibodies.
Disclosure: PLR and SHR are co-applicants on a patent application protecting the detection and/or treatment of diseases associated with autoantibodies to osteoprotegerin. This work was supported by the ECTS Amgen Bone Biology Fellowship (2010) and Coeliac UK/CORE charity (2013).

Table 1 [OC2.3]: Bone mineral density of coeliac patients defined by OPG antibody status

Characteristic	OPG antibody present	OPG antibody absent	p value
Spine BMD T-score	-2.00 (±1.2)	-1.05 (±1.3)	0.02
Spine BMD Z-score	-1.12 (±1.39)	-0.10 (±1.2)	<0.01
Hip BMD T-score	-1.36 (±0.99)	-1.01 (±1.10)	0.29
Hip BMD Z-score	-0.38 (±0.84)	-0.03 (±0.97)	0.24

OC2.4

Mitochondrial DNA Point Mutation is Associated with Lower Bone Mineral Density and Altered Bone Structure in a Matched Case-Control Study

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Background: Mitochondrial dysfunction is associated with several clinical outcomes including diabetes, myopathy, hearing loss and is implicated in the human ageing process. Mitochondrial mutations cause osteoporosis in mouse models. The effect of mitochondrial dysfunction on bone has not been studied in humans.
Methods: We recruited 38 patients (24 female, 14 male) with the mtDNA3243A>G mutation aged 45.8 ±14.9 years. Twenty-three of the cases had diabetes mellitus. Cases were matched with respect to sex, age, height and menopausal status with healthy controls. All participants underwent DXA and HR-pQCT scans. Finite element analysis was used to assess bone strength.
Results: Cases and controls were matched with regard to age, sex and height, but cases had a lower body weight (63.3 vs. 75.7 kg) and higher calcium and vitamin D supplements. Based on DXA, cases had a lower total hip aBMD (0.82 vs. 0.95 g/cm^2, p<0.01), femoral neck aBMD (0.65 vs. 0.80 g/cm^2, p<0.01) and spine aBMD (0.91 vs. 0.98 g/cm^2, p=0.02). Compared to controls, cases had smaller cortical area (radius: 56.0 vs. 64.2 mm^2, p<0.01, tibia: 98.4 vs. 134.6 mm^2, p<0.01), thinner cortices (radius: 0.80 vs. 0.92 mm, p<0.01, tibia: 1.06 vs. 1.29 mm, p<0.01) and lower total bone vBMD (radius: 312.6 vs. 370.8 mg/cm^3, p<0.01, tibia: 275.8 vs. 316.2 mg/cm^3, p<0.01). In cases, cortical density was lower at the radius (888.8 vs. 913.9 mg/cm^3, p=0.02) and trabecular density was lower in tibia (154.1 vs. 176.8 mg/cm^3, p=0.02). In tibia, but not radius, estimated bone stiffness (165.8 vs. 209.4 kN/mm, p<0.01) and failure load (8.5 vs. 10.7 kN, p<0.01) was lower in cases. Hip BMD remained lower in cases after adjusting for weight.
Conclusion: Bone mass, microarchitecture and strength were compromised in patients with mitochondrial dysfunction. Further studies are needed to describe the effects of mitochondrial dysfunction on bone remodelling.
Disclosure: The authors declared no competing interests. Region of Southern Denmark; The A.P. Møller Foundation for the Advancement of Medical Science; Institute of Regional Health Services Research / University of Southern Denmark.

OC2.5

Bone Marrow Lesions Detected by Different Magnetic Resonance Sequences as Potential Biomarkers for Knee Osteoarthritis: Comprehensive Tissue Level Analysis

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Background: MRI-detected bone marrow lesions (BMLs) are associated with symptom severity and structural degeneration in knee osteoarthritis (OA). What BMLs represent at the tissue level is poorly described. The study aim was to characterise the cartilage-subchondral bone features corresponding to BMLs detected using two different MRI sequences for a knee OA cohort.
Methods: Whole tibial plateaus were retrieved from 54 patients (27-female, 27-male), aged 51-86 years, undergoing total knee replacement surgery for OA. To identify BMLs ex-vivo, 3T-MRI scans were performed using T1 and PDFS-weighted sequences. MRI images were used for cartilage volume measurement. Micro-CT was used to assess microstructure of subchondral bone plate (SBP) and trabecular bone (STB). Cartilage and subchondral bone were assessed by OARSI and histopathology. Bone turnover indices were quantitated.
Results: BMLs were detected in 78% of patients (remainder formed No-BML group). Of all BMLs, BML-1 group (BML detected by PDFS only) represented 62%; BML-2 group (BML detected by PDFS and T1) represented 38%. BML-2 had reduced cartilage volume (p=0.007) with increased OARSI degenerative changes (p=0.009) compared to No-BML. BML-2 SBP was thicker and had lower porosity compared with No-BML (p<0.0001). BML-2 STB had higher bone volume (p=0.003), thicker (p=0.002) and more plate-like trabeculae (p=0.0004). SBP and STB osteoid volume and thickness were increased for BML-2 compared to No-BML (p<0.0001). More bone marrow oedema, necrosis and fibrosis was present in BML-2 compared to BML-1 and No-BML (p=0.03).
Conclusion: Knee OA BMLs are associated with loss and degeneration of the overlying cartilage, together with more sclerotic bone morphology. These relationships are more significant for BML-2, suggesting that BML-2 type lesions represent a later stage of OA disease. BMLs detected with specific MRI sequences may act as potential MRI biomarkers for identification of individuals at high risk of progressive OA and inform development and monitoring of new therapies.
Disclosure: The authors declared no competing interests. This work was supported by the National Health and Medical Research Council of Australia (APP1042482).

OC2.6

Gender-Specific Effects of Bisphosphonates on Mortality among Austrian Hip Fracture Patients Aged ≥50 Years

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We retrospectively analysed effects of bisphosphonates (BPs) on mortality in Austrian hip fracture patients. For 31,668 patients ≥50 years sustaining a hip fracture in Austria between July 2008 and December 2010, information on survival with follow-up until June 2011 and on prescription of BPs between July 2007 and June 2011 was available. Using Cox and logistic regression analysis, cumulative all-cause mortality among patients who started treatment before or after fracture was compared with that among age- and sex-matched hip fracture patients without anti-osteoporotic medication. The minimum prescription interval was set at half a year, and matched subjects had to be alive during the prescription interval of his/her assigned treated subject. Compared with female patients receiving no anti-osteoporotic prescription, women who initiated BPs before first fracture (n=8,868) displayed unaltered short-term mortality (hazard ratio [HR] at 90 days after fracture: 0.91 [95%-CI: 0.76-1.09, p=0.30]) but decreased long-term mortality (odds ratios [ORs] at one year and three years' post-fracture, respectively: 0.70 [0.62-0.79, p<0.0001], 0.68 [0.61-0.76, p<0.0001]). Women starting BPs after first fracture (n=3,216) exhibited relative HRs of 0.29 (0.16-0.55, p<0.001) and 0.39 (0.29-0.52, p<0.0001) one year and three years' post-fracture, respectively. For males using BPs already before fracture (n=837), no statistically significant reduction in mortality emerged, however, lowered mortality at one year post-fracture was observed for men treated only after fracture (n=633) (HR 0.12 [0.02-0.88], p<0.05). Among hip fracture patients using BPs, mortality was reduced predominantly in females. The smaller effect of BPs on pre-fracture users' relative to post-fracture users' survival might reflect a selection bias inherent to this observational study with more co-morbidity among BP users than non-users. However, the high extent of mortality reduction found in post-fracture BP users portends a causal relationship with anti-resorptive treatment with BPs.

Disclosure: The authors declared no competing interests.

OC3.1

Identification of Chloride Intracellular Channel Protein 3 as a Novel Gene Affecting Bone Formation

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Osteoporosis is a common skeletal disorder characterised by low bone mass leading to increased bone fragility and fracture susceptibility. Identification of specific factors that stimulate osteoblast differentiation from human mesenchymal stromal cells (hMSCs) may deliver therapeutic targets to treat osteoporosis. The aim is to determine novel factors and mechanisms involved in human osteoblast differentiation. Gene expression profiling was performed on hMSCs differentiated towards osteoblasts or adipocytes using Illumina microarrays. We selected genes that were differentially (2-fold) regulated in the osteogenic versus the adipogenic condition, as well as up- or down-regulated (1.5-fold) versus time point zero. Based on bioinformatic analyses we identified the gene CLIC3 (Chloride Intracellular Channel Protein 3). Lentiviral overexpression of CLIC3 in hMSCs was used to assess the effect osteogenic differentiation. CLIC3 overexpression caused a 34% increase in both alkaline phosphatase activity (p=0.047) and mineralization (p=0.04). Next, we used an *in vivo* human bone formation model where hMSCs lentivirally transduced with the CLIC3 overexpression construct were loaded onto a scaffold (hydroxyapatite-tricalcium-phosphate) and implanted under the skin of NOD-SCID mice and analysed for bone formation after 8 weeks. CLIC3 overexpression led to a 15-fold increase in bone formation (0.33% vs. 5.05% bone area relative to scaffold, p=0.0007). Knockdown of CLIC3 in hMSCs using two short hairpin RNAs against CLIC3 resulted in 89-96% reduction in CLIC3 mRNA expression (p=0.0037 and 0.0026, respectively) and 70-90% less mineralisation (p<0.0001 for both) compared with scrambled control. In conclusion, we successfully identified CLIC3 to be a lineage-specific gene regulating osteoblast differentiation and bone formation. CLIC3 encodes a membrane transport protein that may function in cell growth, vesicle transport, and integrin trafficking. We are currently using pull down and proteomic analysis to investigate the molecular mechanism underlying the CLIC3 control of osteoblast differentiation.

Disclosure: The authors declared no competing interests.

OC3.2

Neuro-Protein CRMP4 Inhibits Bone Formation by Regulating BMP Signalling and Rhoa-FAK Network

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We employed a global gene expression profiling using DNA microarrays to characterise non-canonical osteogenic factors regulating the differentiation of bone marrow skeletal stem cells (marrow stromal stem cells, BMSCs) into osteoblastic cells. We identified CRMP4 (collapsing response mediator protein-4) that was the only member of CRMP1-5 family to be expressed by BMSCs. We found CRMP4, a cytosolic phosphoprotein that mediates Semaphorin-3A effects in neuronal differentiation to be expressed by proliferating chondrocytes and osteoblastic cells and its expression was detected in bone lining osteoblasts in postnatal and adult mouse bones. *In vitro* gain and loss of CRMP4 function in bone marrow stromal cell line ST2 revealed the inhibitory effect of CRMP4 on osteoblast differentiation. Consistently, mice lacking Crmp4 expression displayed significant increased bone mass by 40% compared with wild type controls due to increased trabecular and cortical bone

microarchitecture parameters as measured by micro-CT analysis. Histomorphometic analysis revealed significant increased osteoblast number/bone surface in Crmp4-/- bone with no effect on osteoclastic bone resorption parameters compared with WT controls. Mechanistic studies revealed that increased bone mass in Crmp4-/- mice was associated with upregulation of BMP2-induced osteogenesis in Crmp4-/- osteoblasts (OB) as evidenced by enhanced activation of canonical and non-canonical BMP2 signalling. Furthermore, Crmp4-/-OB exhibited enhanced activation of RhoA/focal adhesion kinase (FAK) signalling that led to cytoskeletal changes associated with increased rate of cell spreading as well as increased cell proliferation rate by increasing the percentage of Crmp4-/- OB in S/G2/M phases of the cell cycle compared with WT OB. The later effect was mediated via inhibiting p21Cip/Waf and upregulating cyclin D1 expression, two targets of RhoA pathway. These findings identify the neuro-protein CRMP4 as a novel negative regulator of bone formation by inhibiting BMP-induced osteogenesis and RhoA-stimulated OB proliferation. Thus, CRMP4 is a new therapeutic target for enhancing bone formation.

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OC3.3

Transgenic Over-Expression of Vitamin D Receptor in Mature Osteoblasts Enhances Catabolic Activities under Dietary Calcium and Phosphorus Restriction

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Osteoblast-specific over-expression of vitamin D receptor (VDR) in a transgenic mouse on a FVB/N genetic background (OSVDR) increases bone volume due to both reduced RANKL-mediated osteoclastic bone resorption and enhanced bone formation. These observations are in contrast to reports of 1,25-dihydroxyvitamin D (1,25D) enhancing osteoclastic bone resorption and inhibiting bone mineralisation. To address this conundrum, 3w female mice with Osteoblast-specific Over-expression of Vitamin D Receptor (VDR) (ObVDR-Tg), Osteoblast-specific VDRKO (ObVDR-KO) and littermate control mice (WT, $VDR^{fl/fl}$) all on a *C57/Bl6* genetic background were fed calcium/phosphorus restricted diet (0.03%Ca, 0.08%Phos; LowCa/P) for 17 weeks and compared with a normal diet (1%Ca, 0.625%Phos; NormCa/P). ObVDR-Tg mice fed the NormCa/P diet demonstrated increased trabecular (64% $P<0.01$) and cortical bone volumes (8%, $P= 0.056$) when compared with WT mice with increased periosteal circumference ($P<0.05$). All mice fed the LowCa/P diet resulted in marked osteopenia with almost total absence of metaphyseal trabecular bone. However, LowCa/P fed ObVDR-Tg mice maintained the increased periosteal circumference, whereas LowCa/P fed ObVDR-KO mice decreased periosteal circumference. Furthermore, LowCa/P fed ObVDR-Tg increased the endosteal circumference, whereas LowCa/P fed ObVDR-KO decreased the endosteal circumference. Interestingly, LowCa/P fed ObVDR-Tg mice exhibited marked intra-cortical porosity and a 22% reduction in cortical osteocyte density. While, serum calcium and phosphorus levels were unaltered in LowCa/P fed ObVDR-Tg mice, serum FGF23 levels were 2-fold lower and serum 1,25D levels were 2-fold higher when compared with WT mice. In addition, RANKL mRNA levels and RANKL:OPG ratio were markedly raised in LowCa/P fed ObVDR-Tg mice. Thus, while overexpression of VDR in osteoblasts can mediate anabolic activities, under conditions of limited dietary calcium and phosphorus, profound bone catabolism prevails possibly due to a lack of appropriate FGF23 feed-back on renal 1,25D synthesis and enhanced RANKL-mediated catabolism.

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OC3.4

Absence of VDR in Mature Osteoclasts Results in Enhanced Resorptive Activity

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Mature osteoclasts express the vitamin D receptor (VDR) and while we have shown that these cells respond to active vitamin D (1,25(OH)2D3), the role for direct activity of vitamin D in regulating osteoclast function is not well understood. To assess the role for VDR-mediated activity in osteoclasts, osteoclast-specific vitamin D receptor knockout mice ($OclVDR^{-/-}$) were generated by mating Cathepsin K^{Cre} with floxed VDR mice ($VDR^{fl/fl}$). Male and female $OclVDR^{-/-}$ and $VDR^{fl/fl}$ littermates were assessed at 6 and 12 weeks of age under normal dietary conditions. In addition, isolated splenocytes from global VDRKO mice or their wild-type (WT) littermates were assessed for osteoclast formation, resorption activity and gene expression under osteoclast-forming conditions. 6w old $OclVDR^{-/-}$ mice demonstrated increased osteoclast surface (Oc.S/BS) in L1 vertebra in both female (+20%, $P<0.05$) and male (+67%, $P<0.05$) mice when compared to $VDR^{fl/fl}$ mice. In $OclVDR^{-/-}$ mice, V-ATPase (V0 subunit) mRNA was increased ($P<0.05$) and Calcitonin Receptor (CTR) mRNA markedly decreased ($P<0.05$). Despite this, biomarkers such as serum X-laps and TRAP5b were not significantly different between $OclVDR^{-/-}$ and $VDR^{fl/fl}$ mice. Furthermore, only males demonstrated a trend for decreased vertebral BV/TV% due to increase trabecular spacing (Tb.Sp) ($P=0.05$). Interestingly, RANKL mRNA levels were significantly decreased suggesting reduced signalling for osteoclastogenesis. VDRKO splenocytes cultured under osteoclastogenic conditions resulted in 2-fold fewer TRAP-positive multinucleated cells ($P<0.05$) compared with WT cells. However, the resorption area on Osteologic™ slides was 3-fold greater per VDRKO osteoclast ($P<0.05$). VDRKO osteoclast CTR mRNA levels associated with reduced Bax/Bcl mRNA ratio were markedly decreased compared with WT suggesting resistance to apoptosis. Thus, while vitamin D

receptor expressed within haematopoietic precursor cells may not be required for differentiation of osteoclasts, the role for VDR in mature osteoclasts appears to be to attenuate resorptive activity.

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OC3.5

Mature Osteoblasts Regulate Vitamin D-Mediated Bone Resorption during Growth and Dietary Calcium/ Phosphorus Restriction

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Active vitamin D (1,25D), bound to the vitamin D receptor (VDR), can directly regulate osteoblast activity modulating bone resorption via induction of RANKL. However, it is somewhat unclear as to which cells of the osteoblast lineage are predominantly responsible for this activity. We have generated mature Osteoblast-VDR Knock Out (mOb-VDRKO) mice using an osteocalcin promoter-Cre to demonstrate the role of VDR-mediated bone resorption in mature osteoblasts during growth and under dietary calcium/phosphorus restriction. 6 week old female mOb-VDRKO mice displayed a pronounced reduction in RANKL mRNA expression, metaphyseal osteoclast surface (OcSur/BS) and serum X-laps. As a consequence, trabecular bone volume (BV/TV%) was increased in the femur (19%, $p<0.05$) and vertebra (21%, $p<0.05$) in comparison to littermate controls. The increase in trabecular bone in female mOb-VDRKO persisted at 12w of age but was absent by 26w of age. By comparison, 6 week old female Osteocyte-specific-VDRKO mice (deletion driven by Dmp1-Cre), exhibited no structural differences in femoral trabecular BV/TV%, and unchanged OcSur/BS. However, vertebral BV/TV% was modestly increased (8%, $p<0.05$) in Oy-VDRKO mice. When 3 week old female mOb-VDRKO mice were subjected to a low calcium (0.03%) and phosphorus diet (0.08%) (LowCa/P) for 3 weeks, serum PTH levels and X-laps levels were approximately 2-fold greater than LowCa/P fed control mice, resulting in the abrogation of the bone phenotype to levels comparable to control mice. When the LowCa/P was continued to 20 weeks of age, higher serum PTH and X-laps levels persisted in mOb-VDRKO mice resulting in deleterious effects on bone including significant intra-cortical porosity. Collectively, these data suggest that mature osteoblasts play a greater role in VDR-mediated bone resorption than osteocytes in young mice. Furthermore, the absence of VDR in mature osteoblasts during calcium/phosphorus restriction results in inappropriately high PTH-mediated bone resorption, possibly through lack of appropriate VDR-mediated bone resorption.

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OC4.1

Unintentional Weight Loss and Fracture: The Global Longitudinal Study of Osteoporosis in Women

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Background: The adverse effects of weight loss on bone mineral density in postmenopausal women are well documented, and increased risk of distal forearm and hip fractures has been reported in studies with average follow-up periods of around 6 years after weight loss. The aim of this study was to investigate the effects of unintentional weight loss in postmenopausal women on the incidence of clinical fractures at multiple sites in the year following weight loss.

Methods: GLOW is an observational longitudinal study of non-institutionalised women aged ≥55 years recruited from 723 primary physician practices in 10 countries. Self-administered questionnaires were mailed and data collected included demographics, medical history, fracture occurrence, medications and weight loss of 10 lb (4.5 kg) or more over the preceding year. Cox models treating weight loss as a time-varying covariate were used to predict fracture in the following survey year, adjusting for factors such as age, prior fracture, co-morbidities, and falls that we have previously shown to be associated with the specific fracture.[1]

Results: Unintentional weight loss of ≥10 lb during the previous 12 months was reported in Year 2 by 3405 (8.0%) of 42,756 and in Year 3 by 3322 (7.7%) of 43,004 women. After adjustment for clinically relevant variables, a significantly increased risk was seen for hip (HR 1.83, 95% CI 1.25–2.69, $p<0.01$) and spine fracture (HR 1.46, 95% CI 1.02–2.09, $p=0.04$) in the year following the unintentional weight loss.

Conclusions: Unintentional weight loss in postmenopausal women is associated with increased risk of hip and spine fracture within the year following weight loss. The rapid time course of this increase in risk has not previously been reported and emphasises the need for prompt fracture risk assessment and appropriate management in women with unintentional bone loss.

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Reference

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OC4.2

Heavy Cannabis use is Associated with Reduced Fat Mass and Increased Fracture Risk but Does Not Influence Bone Density

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Preclinical studies have shown that cannabinoid receptors and their ligands regulate bone metabolism but the clinical significance is unclear. Here we investigated the effects of recreational cannabis use on bone health in the Muirhouse study based in a socially deprived area in the North of Edinburgh. We recruited 263 subjects from the local community through advertisements. Bone density and fat mass were measured on a Hologic QDR4500 densitometer and relevant clinical variables were recorded. Of the 263 individuals recruited 163 (61.9%) were regular cannabis users with a median lifetime use of 20,805 joints (range 4-197,100). The average age of participants was 44±10.3 years and 58% of subjects were female. We divided the population into three groups based on lifetime amount of cannabis taken; none (n=102); light users (4-20,800 joints; n=81) and heavy users (21,000-197,100 joints; n=79). Heavy cannabis users were younger than controls (41.3±1.0 *vs.* 49.6±0.9 years; p<0.001), had a higher dietary calcium intake (1368±104 *vs.* 884±45mg/day; p<0.001); a lower BMI (25.5±0.6 *vs.* 29.3±0.7; p<0.001) and lower fat mass (27.0±9.5 vs 33.8±8.6; p<0.001). The data for moderate cannabis users were intermediate between heavy users and controls (data not shown). Heavy users were significantly more likely to use other illicit drugs (65.8% *vs.* 2.9% for controls; p<0.001). There was no difference in BMD values between cannabis users and controls after adjustment for age, BMI, gender and other relevant variables. Fractures were more common in cannabis users (58% *vs.* 46%; p=0.06), and multiple fractures were significantly more common (10.6% vs 1.9%, p-0.008). Heavy cannabis use is associated with reduced fat mass and an increased risk of fracture, but is not associated with BMD. The differences between mice and men may be due to the complex nature of cannabis, which contains not only THC, a CB1 agonist, but multiple other cannabinoids.

Disclosure: The authors declared no competing interests. This work was supported by Arthritis Research UK.

OC4.3

Significantly Improved Muscle Strength, Running Speed, and Agility in Children with Hypophosphatasia Treated with Asfotase Alfa

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Hypophosphatasia (HPP) is the rare inherited metabolic bone disorder resulting from deficiency in tissue-nonspecific alkaline phosphatase (TNSALP). HPP can cause a spectrum of sequelae in children, including muscle weakness and compromised physical function. 5-12 year-old children treated ≥3 years with asfotase alfa, a recombinant bone-targeted human TNSALP, had improved skeletal mineralisation, growth, and physical function. Here, we report muscle strength and the individual subtests of the Bruininks Oseretsky Test of Motor Proficiency, 2nd Edition (BOT-2) in these children. This ongoing Phase II open-label extension study (6 mg/kg/wk subcutaneous asfotase alfa) assessed bilateral hip and knee extension and flexion, hip abduction, and grip strength by hand-held dynamometry (HHD), reported as percent predicted (%P; right side) for matched, healthy peers. Physical function was evaluated using the BOT-2 Strength subtest (e.g. long jumps, push-ups, etc.), and Running Speed/Agility subtest (e.g. shuttle runs, one-legged hop, etc.). 11/12 patients in the extension study participated in functional testing with last assessment (LA) at 3 years (n=7) or 3.5 years (n=4). Right-side strength (%P) ranged from median (min-max) 32 (9-53; hip extensor) to 60 (21-149; grip) at baseline. Strength in all right-side muscle groups improved at 3 months (P<0.05) except grip, and continued to improve to LA (median 59-98 %P, hip and knee extensor, respectively) (P<0.05). Left side results were similar. BOT-2 Strength scaled score (mean[SD] for healthy peers: 15[5]) improved from median (min-max) 4 (1-13) at baseline to 15 (10-24) at LA (P<0.0001). Median Running Speed/Agility scaled score improved from 3 (1-9) at baseline to 12 (7-19) at LA (P<0.0001). Performance on all BOT-2 subscales improved significantly. These children with HPP had substantial muscle weakness and impaired function at baseline. With asfotase alfa treatment, rapid and continued improvements in strength contributed to significant gains in physical function, which impact ability to perform activities of daily living.

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OC4.4

Odanacatib Anti-Fracture Efficacy And Safety in Postmenopausal Women with Osteoporosis: Results from the Phase III Long-Term Odanacatib Fracture Trial (LOFT)

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LOFT (NCT00529373) is a randomised, double-blind, placebo-controlled, event-driven trial of odanacatib (ODN), an oral selective inhibitor of cathepsin K. Postmenopausal women ≥65 years with bone mineral density (BMD) T-score ≤–2.5 at

total hip (TH) or femoral neck (FN), or with prior radiographic vertebral fracture (VFx) and T-score ≤–1.5 at TH or FN, were randomised to ODN 50 mg/week or placebo. Patients received vitamin D (5600 IU/week), plus calcium to achieve intake of 1200 mg/day. Primary endpoints were: new/worsening morphometric VFx; hip fractures; non-vertebral fractures. Secondary endpoints included clinical VFx; BMD; bone turnover markers. Safety/tolerability measures included adjudicated adverse events (AEs) of interest. 16,713 women were randomised; 16,071 were included in analysis. Mean age was 72.8 years; 46.5% had prior radiographic VFx. Mean BMD T-scores were: lumbar spine (LS) –2.7; TH –2.4; FN –2.7. Mean follow-up was 34.5 months. Versus placebo, ODN treatment resulted in relative risk reductions of: 54% for new/worsening morphometric VFx; 47% for hip fractures; 23% for non-vertebral fractures; 72% for clinical VFx (p<0.001). ODN treatment led to progressive increases over 5 years in BMD at LS and TH: 11.2% and 9.5%, respectively, versus placebo. The incidence of AEs and serious AEs overall did not differ meaningfully between groups. There were 271 deaths reported in the ODN group and 242 on placebo (hazard ratio 1.13 [95% CI: 0.95, 1.35]); this numeric imbalance in mortality did not appear related to a particular reported cause of death. Adjudicated morphea-like skin lesions and femoral shaft fractures (including those with atypical features) occurred in small numbers of patients, more commonly with ODN than placebo. No cases of osteonecrosis of the jaw were reported. Major cardiovascular events overall were generally balanced; however, there were numerically more adjudicated strokes with ODN than with placebo. Final blinded adjudication of major cardiovascular events is ongoing.

Disclosure: Authors conflicts of interest for Merck include: consulting fees (MRM, BL, SP, KS, HB); grants (MRM, BL, KS, HB); royalties (MRM); participation in speakers bureaux (MRM, BL); employee (AR-F, DC, CAD, RM, AS, BBS, KDK, NV, ALo); former employee (ALe). This study was sponsored by Merck & Co., Inc.

OC4.5

Tracking of 25-Hydroxyvitamin D Status in Pregnant Women

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Background: When assessed in pregnancy studies, 25(OH)D is usually measured only once. However, it is unknown whether the ranking of an individual's 25(OH)D is maintained across pregnancy, which crosses several seasons. We therefore assessed the tracking of 25(OH)D from early to late pregnancy in a prospective mother-offspring study, the Southampton Women's Survey.

Methods: At 14 and 34 weeks gestation, serum 25(OH)D was measured, and diet and lifestyle questionnaires completed. We modelled seasonal variation in 25(OH)D separately for each time point using Fourier transformations, and then calculated the difference between actual 25(OH)D and the modelled value corresponding to the sampling date for each individual [denoted $25(OH)D_{dev}$]. We used Spearman's rank correlation to test tracking of $25(OH)D_{dev}$ from 14 to 34 weeks gestation. Multivariate linear regression was used to determine factors associated with alterations in an individual's $25(OH)D_{dev}$ ranking.

Results: 25(OH)D was available in 2060 and 2332 women at 14 and 34 weeks, respectively, with 1756 women included at both gestations. $25(OH)D_{dev}$ tracked moderately from 14 to 34 weeks (r=0.57, p<0.0001), although some women had marked changes in $25(OH)D_{dev}$ across pregnancy (median: -0.8; range: -150.1 to 129.6nmol/l). 25(OH)D tended to fall with greater pregnancy weight gain ($25(OH)D_{dev}$ β=-0.4nmol/l per kg, p=0.02), and to rise with greater strenuous activity in late pregnancy (β=1.0nmol/l per hour/week, p=0.03). Vitamin D supplementation was the strongest influence on tracking: compared with women who never used supplements, discontinuing supplementation after 14 weeks was associated with negative change in $25(OH)_{dev}$ (β=-7.2nmol/l, p<0.001), whereas commencing (β=12.2nmol/l, p<0.001) or continuing (β=8.0nmol/l, p<0.001) supplementation were positively associated.

Conclusion: Stability of an individual's gestational 25(OH)D relative to the population is modest, and affected by weight changes, activity levels and vitamin D supplementation. These findings may explain some of the observed heterogeneity in studies relating maternal vitamin D status to offspring health.

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OC4.6

Meta-Analysis of Observational Studies on the Effect of Incretin Treatment on Fracture Risk

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Background: In Europe, approximately 60 million patients suffer from type 2 diabetes mellitus (T2DM). T2DM patients are at increased risk of fracture. Incretin agents are used to treat T2DM, and they include two classes: glucagon-like-peptide 1 receptor agonists (GLP1-RAs) and dipeptidyl peptidase-4

inhibitors (DPP4-Is). Currently, there is no data available from electronic healthcare databases. The objective of this study was to evaluate the association between incretin agents and risk of fracture.
Methods: We used data from the UK Clinical Practice Research Datalink (CPRD), the world's largest primary care database, representative for the total UK population (2007-2012, n=13 million) and from the full country of Denmark (2007-2011, n=5.5 million). We used a cohort design and Cox regression analysis with CPRD data and a case-control study with conditional logistical regression in Denmark (which comprised all patients with a first fracture matched to controls). We compared current incretin use with non-use. A meta-analysis extracted hazard- (HRs) and odds ratios and their corresponding 95% confidence intervals (CIs) using generic inverse variance methods assuming a random effects model.
Results: Use of incretin agents was not associated with fracture risk in both countries (adj. pooled risk ratio DPP4-I and GLP1-ra: 1.01; 95% CI 0.92 – 1.12, 1.03; 95% CI 0.87 – 1.22, respectively). Increasing cumulative dose did not further decrease risk of fracture yielding adj. HRs of 1.07; 95% CI 0.90 – 1.27 (0-18.2 mg) adj. HR 0.84; 95% CI 0.67 – 1.06 (18.3-36.5 mg) adj. HR 1.05; 95% CI 0.81 – 1.37 (36.6-54.7 mg), adj. HR 0.97; 95% CI 0.78 – 1.20 (> 54.7 mg).
Discussion: Use of incretin agents was not associated with fracture risk in both countries, and higher cumulative dosages did not result in an inverse association. Our results do not support the conduct of further clinical research to study beneficial effects of incretin agents on fracture risk.
Disclosure: The authors declared no competing interests.

OC5.1
Inhibition of the Interleukin-6-Induced STAT3 Signalling Pathway is Chondroprotective
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Background: High levels of interleukin-6 (IL-6) have been found in the synovial fluid of patients with osteoarthritis (OA), suggesting that IL-6 may be involved in the pathogenesis of OA. The objectives were to investigate the effects of IL-6 in chondrocytes and to determine its main signalling pathways; and to study the impact of IL-6 inhibition in an experimental mice model of OA.
Methods: The effects of IL-6 (10-50-100 ng/mL) were determined in vitro (primary culture of mouse chondrocytes) and *ex vivo* (mouse femoral head articular cartilage). Proteoglycan content (Alcian blue and Safranin O staining, DMM blue assay), expression of catabolic factors (qPCR, Western Blot, immunostaining), NO and PGE2 production and apoptosis (TUNEL assay) were evaluated. IL-6-induced signalling pathways were determined by western blot. The impact of STAT3 blockade was investigated using a specific inhibitor – Stattic – ex vivo and in a mice model of OA induced by destabilisation of the medial meniscus (DMM).
Results: *In vitro* and *ex vivo*, IL-6 dose-dependently induced a dramatic loss of proteoglycan content through an increase in the expression of MMP3, MMP13, ADAMTS4 and ADAMTS5. By contrast, IL-6 had no effect on col2, aggrecan, col10 or VEGF. IL-6 induced chondrocytes apoptosis without increasing NO or PGE2 production. Inhibition of STAT3 by Stattic counteracted the catabolic and pro-apoptotic effects of IL-6 *ex vivo*. Finally, we orally administrated either Stattic (25 mg/kg/2d) or a saline for 6 weeks in C57/Bl6 mice (n=18) subjected to DMM. The severity of the OA lesions as assessed with the OARSI histological score was significantly lower in the Stattic group: 2.65 ± 1.44 vs. 4.5 ± 0.93 (p=0,004).
Conclusion: Our findings indicate that IL-6 has numerous catabolic effects in cartilage, mainly mediated by STAT3. STAT3 blockade protects against DMM-induced OA in mice, suggesting that IL-6 might be a promising therapeutic target in OA.
Disclosure: The authors declared no competing interests. This work was supported by the Société Française de Rhumatologie.

OC5.2
Genetic Variants in the SUPT3H-RUNX2 Locus Confer Susceptibility for Bone and Cartilage Related Disorders via Long-Range Regulation Of RUNX2
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Genome-wide association studies (GWAS) have identified in total 6 independent SNPs within the 5' region of the *RUNX2* gene to be robustly associated with 5 different cartilage and bone related phenotypes. We aim to elucidate the effect of the identified SNPs on the regulation and expression of *RUNX2* and how these confer susceptibility to cartilage and bone related disorders, such as osteoarthritis and osteoporosis. Independent GWAS signals and SNPs in LD with the GWAS loci were identified with GCTA conditional joint analysis, the SNAP tool, and HaploReg (V2.2, Broad Institute). GWAS SNPs and SNPs in high LD were analysed for enrichment in genomic regulatory regions, and co-location with DNA binding proteins using data from the ENCODE-Project, Roadmap epigenetics project, and the FANTOM5 database. In human cartilage explants we measured *RUNX2* expression by RNA sequencing, CTCF-DNA binding by ChIP-qPCR and preformed eQTL analysis to determine the effect of the SNPs on gene expression. We found 6 genetically independent GWAS signals to co-localise to regions with enrichment of active enhancer markers, H3K4me1, H3K27ac, DNase1 hypersensitivity enrichment and bi-directional CAGE reads, in osteoblast and chondrogenic cells. The BMD associated SNP located ~700 kb away from the *RUNX2* promoter, had a significant effect ($p<0.05$) on *RUNX2* gene expression in human cartilage. In addition, we observed that when we stimulated *RUNX2* expression in human chondrocytes by TGFβ stimulation, there is an increase in binding of the chromatin-loop mediating protein, CTCF, near the *RUNX2* promoter. We have found that variants in the *SUPT3H-RUNX2* locus associated to cartilage and bone phenotypes are located in gene regulatory regions, and affect *RUNX2* gene expression. We hypothesise that the SNPs are localised in long-range enhancers which, mediated by a CTCF

chromatin-loop to the *RUNX2* promoters, regulate *RUNX2* gene expression in bone and cartilage development.
Disclosure: The authors declared no competing interests. This work was supported by the Netherlands organisation for scientific research (NWO) VIDI-scheme.

OC5.3
MUC1 in Osteoblasts Balances Osteogenesis and Angiogenesis under Hypoxia
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It is critical that bone formation and angiogenesis are tightly coordinated during bone development and fracture healing. Oxygen tension impacts both processes. Previously we demonstrated that hypoxia limits osteoblast differentiation/mineralisation and strongly induces mucin1 (MUC1) expression in human osteoblasts. Expression of MUC1 is positively associated with hypoxia-driven angiogenesis. Thereby MUC1 is a likely candidate to control both osteogenesis and angiogenesis. We investigated MUC1 function in osteoblasts and its role in the interaction between bone formation and angiogenesis. Hypoxia (2% O_2)-induced inhibition of osteoblast differentiation (Alkaline phosphatase activity -64%) and mineralisation (-89%) was prevented by blocking MUC1 function using either a specific inhibitor (GO-201) or 2 shRNAs. This was supported by studies using osteoblasts cultured from bone marrow of *Muc1* knockout mice. Conditioned medium of osteoblasts cultured under hypoxia (HCM) stimulated endothelial migration (+80%) and angiogenesis (+150%), which was prevented by blocking MUC1 in osteoblasts using GO-201 or shRNA. Mass spectrometry analysis identified among others vascular endothelial growth factor (VEGF)-A and macrophage migration inhibitory factor (MIF) to be present in control HCM but not in HCM of osteoblasts treated with GO-201 and shRNA. VEGF neutralising antibody or MIF inhibitor 4-IPP prevented HCM-induced endothelial morphogenesis. HCM induced nitric oxide (NO) production (1.8 fold increase) in human endothelial cells and inhibition of NO production blocked the angiogenic effect of HCM. Finally, it was shown that nuclear translocation of the MUC1 cytoplasmic tail in osteoblasts is essential for the effects observed. In conclusion, we demonstrate that MUC1 in osteoblasts is at the crossroad of oxygen control of osteoblast differentiation/mineralisation and angiogenesis. The level and nuclear translocation of MUC1 in osteoblasts determines whether under hypoxia either bone formation or angiogenesis prevails. Thereby, these data contribute to the molecular understanding of the balance between osteogenesis and angiogenesis in bone development and fracture repair.
Disclosure: The authors declared no competing interests. This work was supported by the European Union (PIRSES-GA-2011-295181).

OC5.4
Osteoblast-Secreted Extracellular Vesicles Stimulate the Expansion of CD34⁺ Human Umbilical Cord Blood Cells
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Umbilical cord blood (UCB) is increasingly used in haematopoietic stem cell (HSC) transplantations; however, the low cell numbers are still remaining as a limiting factor for proper engraftment. Osteoblasts play important roles in regulating HSC self-renewal and differentiation. Recently, extracellular vesicles (EVs) have been implicated in stem cell fate regulation via horizontal transfer of proteins and nucleic acids between cells. In this study, we focused on the characterisation of osteoblast EVs and investigated their potential in *ex vivo* expansion of $CD34^+$ UCB cells for clinical use. We used human pre-osteoblasts (SV-HFO cells) to isolate EVs, and characterised EVs by electron microscopy, proteomics, and RNA sequencing, and investigated their functional effect on human $CD34^+$ UCB cells by qPCR and flow cytometry. Characterisation analyses demonstrated that osteoblast EVs are heterogenic in size, contain novel osteoblast EV proteins primarily linked to ribosomal activity and RNA processing, and are enriched with small RNAs. Treatment of $CD34^+$ UCB cells with osteoblast EVs led to donor-dependent 2-3-fold expansion ($p < 0.01$) of the $CD34^+$ expressing progenitors in 10 days. MicroRNA profiling demonstrated that osteoblast EVs contain abundant amounts of miR-29a, one of the key regulators of early haematopoiesis. Interestingly, EVs treatment led to the two-fold down-regulation ($p < 0.01$) of HBP1, a miR-29a target that has been shown to be a cell cycle inhibitor, in $CD34^+$ UCB cells. Consequently, cell cycle analysis showed that EVs stimulated progression from G0/G1 to S/G2 phase ($p < 0.05$), which may explain the mechanism by which EVs stimulate UCB cell expansion. Finally, EV-expanded $CD34^+$ UCB cells showed good clonogenicity and differentiation potential *in vitro* and successful engraftment in a NOD/SCID-IL2Rγ (NSG mice) xenograft model *in vivo*. In this study, we demonstrated that osteoblasts secrete EVs that expand UCB cells *ex vivo*, and uncovered the first clues that contributed to the understanding of EV function.
Disclosure: The authors declared no competing interests. Erasmus MC Stem Cell and Regenerative Medicine Institute.

OC5.5
Sclerostin Depletion and its Effect on Fracture Healing in the Mouse Model
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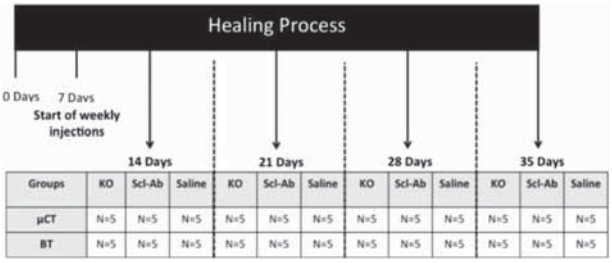
Background: Sclerostin is a secreted glycoprotein that interacts with LRP5 receptor on osteoblasts and inhibits the intracellular Wnt signalling pathway, leading to decreased bone formation. When sclerostin is inactivated bone formation is therefore stimulated. This stimulation has been proven in



Groups	KO	Scl-Ab	Saline	KO	Scl-Ab	Saline	KO	Scl-Ab	Saline	KO	Scl-Ab	Saline
µCT	N=5	N=5	N=5	N=5	N=5	N=5	N=5	N=5	N=5	N=5	N=5	N=5
BT	N=5	N=5	N=5	N=5	N=5	N=5	N=5	N=5	N=5	N=5	N=5	N=5

Figure 1. Fracture model protocol.

fracture studies, which showed that sclerostin deficient mice have larger and stronger calluses with accelerated fracture healing, both in sclerostin knockout and sclerostin antibody injection models. These observations suggest that sclerostin inhibition and depletion show improved and accelerated fracture healing, but the effect of these two mechanisms have not been compared to assess the accurate effect of the Scl-Ab injections. Therefore we designed a study to compare the effect of sclerostin depletion (sclerostin knockout) and inhibition (Scl-Ab injection).

Methods: Ten-week-old male SOST knockout (KO) (N=20) and wild-type (WT) (N=40) mice underwent insertion of a tibial intramedullary pin after which a mid-shaft tibial osteotomy was performed. The mice were divided into three groups: SOST KO (N=20), WT with Scl-Ab injection (N=20) and WT with saline injection (N=20). The Scl-Ab group received an intravenous dose of 100mg/kg weekly starting on day 7. Each group was managed and sacrificed according to the specified protocol (Figure 1). For data analysis, one-way ANOVA (Analysis Of Variance) was performed followed by Tukey's post hoc test at each time point. P values<0.05 were considered statistically significant.

Results: Both Scl-Ab and KO groups showed significantly increased trabecular BV/TV (bone volume/ total volume) at the fracture site (mid-shaft of the tibia) compared to the saline group at all time points and also showed no significant difference between them at all time points (except at 28 days postoperative) (Figure 2). On biomechanical testing the Scl-Ab and KO groups showed significant increased strength in stiffness at days 14, 28 and 35 compared to the saline group (Figure 3A). Concerning ultimate force and work to failure the KO group showed significant increase in the force required compared to both the Scl-Ab and saline groups at 21,28 and 35 days. While the Scl-Ab group showed increased forced required to fracture the callus compared to the saline group at these time points, but this was only significant for work to failure at 28 days (Figure 3B, D).

Conclusion: Scl-Ab injections showed promising results, which were comparable to the complete depletion of sclerostin, especially at earlier stages of the healing process. In addition, our results indicate that sclerostin antibody exerts its greatest effect in the earlier stages of fracture healing (days 14 and 21), after which the healing process plateaus and thus completing this process at an earlier time point. Further re-

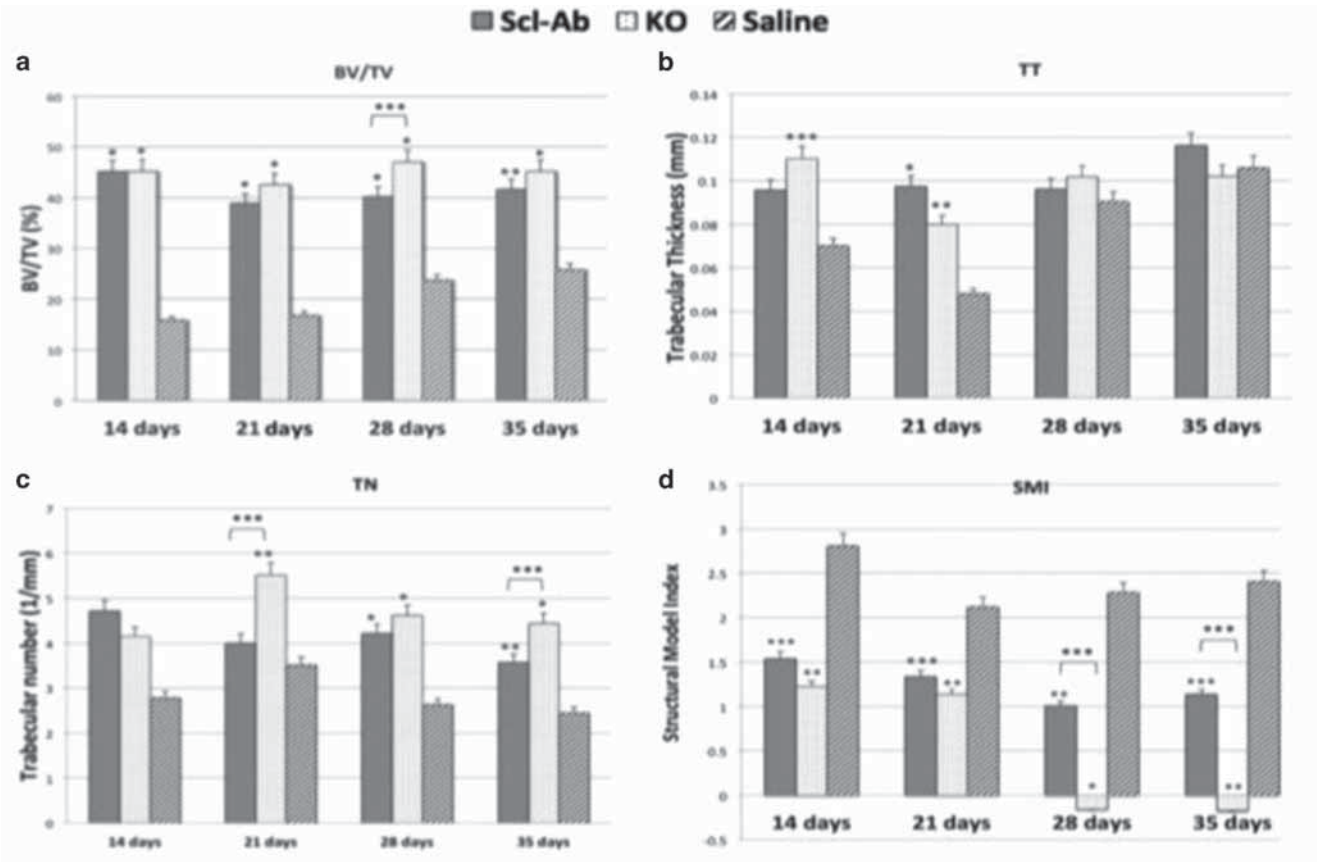


Figure 2: MicroCT results across all time points. Data presented as mean and standard error of the mean (*P<0.001, **P<0.01, ***p<0.05).
Abrev: BV/TV; bone volume/total volume, Tb.Th; trabecular thickness, Tb.N; trabecular number, SMI; structural model index).

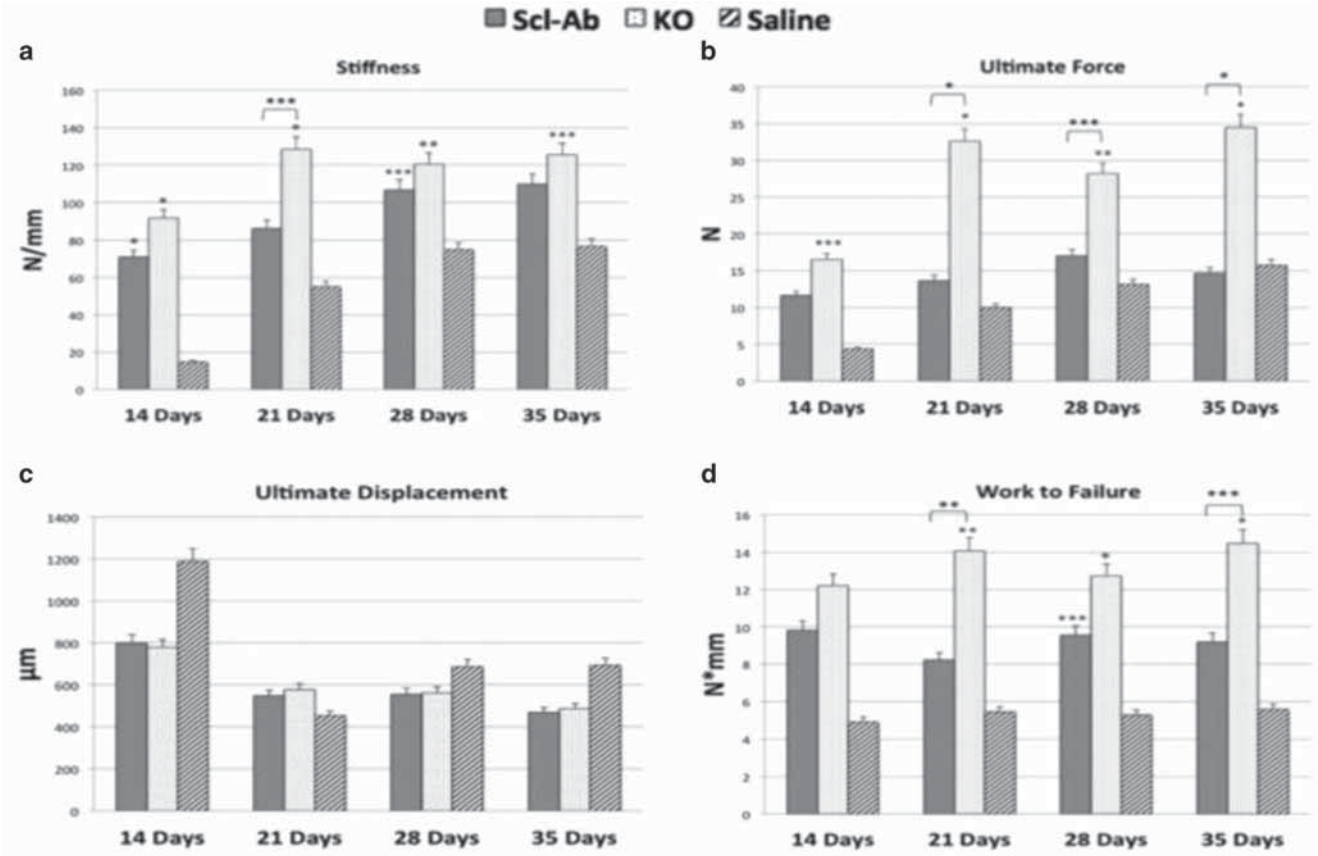


Figure 3: Biomechanical testing results across all time points. Data presented as mean and standard error of the mean (*P<0.001, **P<0.01, ***p<0.05).
Abrev: N; newton

search into accurate dosage and adequate timing of administration is required before these promising results can be implicated as a modality for accelerating fracture healing in humans and management of delayed / nonunion.
Disclosure: The authors declared no competing interests.

OC6.1
Secular Change in Fracture Incidence is Not Associated with Better Post-Fracture Outcomes: a Time-Trend Comparison between Two Birth Cohorts
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During the last decade, hip fracture incidence declined and life expectancy improved. However, it is unclear whether the outcomes following osteoporotic fracture have also changed. The aim of this study was to compare re-fracture risk and excess mortality following osteoporotic fracture between two birth cohorts and over 2 time intervals 1989-1999 and 2000-2010. Study participants comprised women and men 60+ participating into DOES1 (born before 1930) and DOES2 (born after 1930). All fractures excluding head, fingers and toes were recorded between 1989 and 2010. Age-standardised fracture incidence and mortality rates were calculated in two time intervals: 1989-1999 (for DOES1) and 2000-2010 (for DOES2). The difference in excess mortality between the 2 cohorts was assessed using standardised mortality ratios (SMR) calculated for each study cohort using time-specific population mortality rates. The prevalence of osteoporosis declined and the level of treatment increased significantly in DOES 2 compared to DOES 1. Fracture incidence declined by ~10% in both genders, however, not significantly. Interestingly, re-fracture risk was similar for DOES1 and DOES2 [women age-adjusted RR 2.0 (95% CI, 1.6-2.5) in DOES1 and 1.9 (95% CI, 1.7- 2.3) in DOES2 and men, 3.5 (95% CI, 2.7-4.8) in DOES1 and 3.4 (95% CI, 2.7- 4.5) in DOES2]. Crude mortality rates decreased during study follow-up. However, after taking into account the difference in general population life expectancy during the 2 study periods, the excess mortality post-fracture was similar [women, SMR 2.1 (95% CI, 1.7- 2.6) in DOES1 and 1.7 (95% CI, 1.2- 2.4) in DOES2, and men, 1.9 (95% CI, 1.5- 2.5) in DOES1 and 1.9 (95% CI, 1.3- 2.7) in DOES2]. Thus despite a reduction in the prevalence of osteoporosis and improvement in treatment uptake over the last 2 decades, re-fracture risk and fracture-associated excess mortality was similar. The reasons for this deserve urgent exploration.
Disclosure: The authors declared no competing interests. This work was supported by the National Health Medical Research Council Australia (NHMRC project ID; DB 1073430, JRC 1008219, TVN, JRC and JAE 1070187).

OC6.2

Intronic Mutations in the *TCIRG1* Gene Cause Human Autosomal Recessive Osteopetrosis

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Autosomal Recessive Osteopetrosis (ARO) is a rare genetic bone disease with genotypic and phenotypic heterogeneity, sometimes translating into delayed diagnosis and treatment; in particular, intermediate cases often constitute a diagnostic challenge. Mutations in the TCIRG1 gene are responsible for more than 50% of ARO cases, and a wide range of molecular defects have been found. Here we describe the identification of 4 different single nucleotide changes in intron 15 in 5 patients from 4 unrelated families. These novel mutations were in the middle of a 368 nucleotide long intron, far from the canonical splice sites; therefore, they were missed by standard gene amplification and sequencing, focused on exons and exon-intron boundaries, and went ignored by exome sequencing. In 3 out of 5 patients, by cloning and sequencing a number of independent cDNA clones covering exons 14 to 17, we demonstrated a reduced splicing efficiency, which did not completely abrogate the production of the normal transcript. In conclusion, we identified an intronic region in the TCIRG1 gene which seems to be prone to splicing mutations. These molecular defects allow the production of a small amount of protein sufficient to dampen the severe phenotype usually associated to TCIRG1 mutations. Indeed, the patients bearing these variants displayed a different level of severity of the disease, with 3 out of 5 reaching adulthood with a mild presentation. On this basis, we suggest the analysis of the TCIRG1 gene is appropriate not only in the molecular work up of severe patients, but also of intermediate cases. In addition, our results demonstrate that standard protocols for gene testing are likely to be revised. In particular, intron 15 should be included in the routine sequencing of the TCIRG1 gene; more in general, the effect of intronic changes in genes associated with osteopetrosis should be carefully evaluated.

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OC6.3

The Effects of Daily Teriparatide on the Spine and Femoral Strength Assessed by a Finite Element Analysis of Clinical Computed Tomography Scans in Rheumatoid Arthritis Patients

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Background: To evaluate the quantitative effects in RA patients who are treated with TPTD after 12 months by several methods.

Methods: Twenty-seven RA patients were enrolled in this prospective study. All patients who were receiving TPTD were evaluated according to changes in two bone turnover markers (serum procollagen type 1 N-terminal propeptide [P1NP] and, tartrate-resistant acid phosphatase-5b [TRACP-5b]) from baseline to 1, 3, 6 and 12 months. They were assessed according to bone mineral density (BMD) by dual X-ray absorptiometry (DXA) and bone strength by quantitative computed tomography (CT) at baseline. They were re-evaluated after 12 months. Nonlinear finite element analysis (FEA) was performed on the CT scans to compute an estimate of spinal and femoral-fall configuration predicted bone strength (PBS) by FEA.

Results: Patients were aged 68.2 years and their duration of symptoms was 12.2 years. The majority of subjects had moderate disease activity (mean baseline 28-joint Disease Activity Score, 3.0±1.3). The mHAQ scores median were 0.8. On average, PINP (baseline, 1, 3 and 6 months) was 42, 141, 144 and 153μg/l, TRACP-5b was 423, 527, 583 and 601 mU/dl. Patients had significantly greater levels of serum PINP and TRACP-5b ($p<0.05$ compared with baseline) at all points measured. On average, BMD-spine (baseline and 12 months) was 0.89, 0.94 g/cm^2 ($p<0.01$) (median change 6.3%), BMD-femoral neck was 0.62, 0.62 g/cm^2 ($p=0.31$) (median change 1.4%), PBS-spine was 3508, 4070 N ($p<0.01$) (mean change 19.8%), and femoral PBS- fall was 1428, 1441 N ($p=0.2$) (mean change 1.6%).

Conclusions: Our results show that TPTD can increase BMD and FEA on RA patients, and indicate that bone loss can be prevented in patients with RA by TPTD. FEA should detect changes of TPTD effects more sensitive than DXA. We will have to follow these effects in longer term.

Disclosure: The authors declared no competing interests.

OC6.4

Hyponatraemia is Prevalent and Associated with 30-Day Mortality in Hip Fracture Patients

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Background: The relationship between bone metabolism and plasma sodium levels has lately gained increasing interest as hyponatraemia has been linked to both increased risk of osteoporosis and fractures. The aim of this study was examine the frequency of hypo- and hypernatraemia in patients admitted with a fractured hip and the association with 30-day mortality in these patients.
Methods: A database of all surgically treated hip fracture patients admitted to our hospital between January 1996 and November 2013 was searched for all patients aged 60 years or above. 7755 patients were identified and a search for plasma sodium levels for these patients was conducted in the hospitals laboratory system. 7644 (98.6%) had a preoperative admission plasma sodium measurement and composed the study cohort. Comorbidity was included in the form of Charlson Comorbidity Index, which was calculated based on data from the Danish National Patient Registry. Hyponatraemia was defined as $[Na^+]<135$ mmol/l and hypernatraemia as $[Na^+]>145$mmol/l.
Results: The patients had a mean age of 82.5 (SD 8.5) years and 76.5% (5845/7644) were female. 19.0% (1455/7644) were hyponatraemic, 1.6% (123/7644) were hypernatraemic and 79.4% (6066/7644) were normonatraemic on admission. There was an increased 30-day mortality rate for patients with hyponatraemia (12.1%, p=0.008 (chi-square)) and hypernatraemia (16.3%, p=0.02 (chi-square)) compared to normonatraemic patients (9.7%). The hazard ratios for 30-day mortality were 1.26 [1.06;1.49] (unadjusted) and 1.35 [1.14;1.60] (adjusted for sex, age and comorbidity) for hyponatraemic patients and 1.74 [1.12;2.72] (unadjusted) and 1.76 [1.13;2.78] (adjusted for sex, age and comorbidity) for hypernatraemic patients.
Conclusion: The study showed that the prevalence of hyponatraemia in hip fracture patients was high. Furthermore, patients with decreased or elevated plasma sodium levels had an increased mortality rate. Disturbances in plasma sodium levels may itself cause increased mortality but could also be a surrogate marker for frailty in these patients.
Disclosure: The authors declared no competing interests.

OC6.5

The Calcineurin Inhibitor Tacrolimus as a New Therapy in Severe Cherubism

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Cherubism is a rare genetic disorder characterised by extensive growth of a bilateral granuloma of the jaws, resulting in facial disfigurement. Cherubism is caused by gain-of-function mutations in the *SH3BP2* gene, leading to over-activation of NFATc1-dependent osteoclastogenesis. Recent findings in human and mouse cherubism suggested that calcineurin inhibitors might be drug candidates in cherubism medical treatment. A 4-year-old boy with aggressive cherubism was treated with the calcineurin inhibitor tacrolimus for one year, and clinical, radiological, and molecular data were obtained. Immunohistological analysis was performed to compare pre- and post-operative NFATc1 staining and TRAP activity. Real-time PCR was performed to analyse the relative expression levels of *OPG* and *RANKL*. After tacrolimus therapy, the patient showed significant clinical improvement, including stabilisation of jaw size and intra-osseous osteogenesis. Immunohistological analyses on granuloma showed that tacrolimus caused a significant reduction in the number of TRAP positive osteoclasts and NFATc1 nuclear staining in multinucleated giant-cells. Molecular analysis showed that tacrolimus treatment also resulted in increased *OPG* expression. We present the first case of effective medical therapy in cherubism. Tacrolimus enhanced bone formation by stimulating osteogenesis and inhibiting osteoclastogenesis.
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OC6.6

Blood Circulating miRNAs are Indicative of Skeletal Fractures in Postmenopausal Women with and Without Type 2 Diabetes and may be Promising Candidates for General Fracture Risk Prediction

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Fracture risk in type-2-diabetes (T2D) and postmenopausal osteoporosis is routinely assessed with FRAX or DXA although these methods show limitations especially in T2Diabetics. Novel, general applicable biomarkers are therefore desirable. MicroRNAs (miRNAs) are secreted into the circulation from cells of various tissues proportional to local disease severity and were recently found to be crucial to bone homeostasis ("osteomiRs") and T2D aetiology. The objective of this study was to analyse circulating miRNAs in a well-characterised study of postmenopausal and diabetic osteoporosis and to evaluate their utility for general fracture risk assessment. MiRNA-qPCR-arrays and differential-expression-analysis of 153 miRNAs were performed from 74 serum samples drawn from postmenopausal T2D women with (DMFX, n=19) and without fracture history (DM, n=19) as well as from non-diabetic women with (Fx, n=19) and without fracture history (Co, n=17). Group-wise non-parametric statistical comparisons were used with BH-adjustment of p-values for multiple testing. Circulating miRNAs exhibiting significant differences were then used for building multi-parametric models to differentiate fracture patients from controls. Cumulative ROC analyses yielded AUC-values of 0.978 for Fx/Co-comparisons (based on 4 miRNAs) and 0.933 for DMFX/DM-comparisons (based on 4 miRNAs). Interestingly, the 4 highly discriminative miRNAs of each comparison did not overlap. We found that some of them have been previously described as "osteomiRs", such as miR-155-5p, an initiator of osteoclastogenic differentiation, or miR 96-5p, an osteocyte negative marker. All remaining ones had not been previously characterised (e.g. miR-188-3p and miR-203a) yet. Therefore, additional *in vitro* tests were performed, to characterise their (anti)-osteogenic activity. Our data provide first evidence that certain circulating miRNA levels are indicative of fragility fractures in postmenopausal women with and without diabetes and may be novel candidates for general fracture risk screenings. Future studies will elucidate if this knowledge can be used to improve current diagnostic techniques to predict fracture risk and therapy response in elderly women.

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CABS ORAL COMMUNICATIONS

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CABS OC1.1

The Sesquiterpene Lactone Parthenolide Protects Against Cancer Cell-Induced Osteolysis by Inhibiting Osteoclast Formation and Promoting Osteoblast Differentiation

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The NFκB pathway plays an important role in inflammation and bone remodelling. The sesquiterpene lactone Parthenolide (PTN) is a potent NFκB inhibitor, and previous studies showed that PTN reduces osteolysis associated with breast cancer (Idris *et al*, 2009). Here, we took advantage of an *in vivo* supracalvarial injection, *ex vivo* mouse calvarial organ and *in vitro* co-culture models to assess the cell-autonomous effects of PTN on cancer cell-induced osteolysis. MicroCT analysis of calvarial bone showed that human MDA-231, mouse 4T1 and rat MLL cancer cells caused osteolysis when co-cultured with mouse calvaria, and these effects were significantly reduced by PTN (1 μM; MDA-231, 56%; 4T1, 62%; MLL, 36%, increase in BV/TV, $p<0.05$). Treatment of osteoblasts with PTN (1 μM) increased alkaline phosphatase levels (62% increase, $p<0.01$), enhanced bone nodule formation (42% increase, $p<0.01$) and reduced the ability of MDA-231 and 4T1 conditioned medium to enhance osteoblast support for osteoclastogenesis (MDA-231, 38% and 4T1, 24%; reduction, $p<0.05$) and induce mRNA expression of RANKL (85% reduction, $p<0.01$) and OPG (76% reduction, $p<0.01$). Moreover, PTN also inhibited RANKL (IC50; 1.6 μM), MDA-231 (IC50; 1.1 μM), 4T1 (IC50; 1.2 μM) and MLL (IC50; 1.4 μM) induced osteoclast formation in a dose dependent manner. Finally, intraperitoneal administration of PTN (1 mg/kg/day) in adult immune-competent mice prior to supracalvarial injection of MDA-231 conditioned medium caused a significant reduction of osteolysis (37% increase in BV/TV, $p<0.05$). This effect was found to be strongly associated with inhibition of NFκB-mediated pro-inflammatory actions of the bone- and tumour-derived factors RANKL, TGFβ, IL-8 and CXCL1 that alter the balance of osteoblasts and osteoclasts in bone metastatic microenvironment. Collectively, our findings suggest that, due to the combined anti-resorptive and osteoanabolic effects, PTN, or similar sesquiterpene lactones currently in clinical trials for advanced solid tumors, has potential as a promising therapeutic agent for the treatment of osteolytic bone disease.
Disclosure: The authors declared no competing interests.

CABS OC1.2

Breast Cancer Cells Compete for the Space in the Bone Metastatic Niche

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During dissemination to the skeleton, breast cancer cells are proposed to localise in a putative metastatic niche, situated in close proximity to the endosteal bone surface. To assess whether tumour cells compete for the space in this niche, we mapped the number and location of tumour cells in the tibia 5/12 days following injection of human breast cancer cells in immunocompromised mice. Female 12-week old BALB/c nude mice were injected i.v. with $1x10^5$ MDA-MB-231-IV breast cancer cells labelled with a lipophilic dye (Vybrant-DiD/Vybrant-CM-DiI). The number and location of tumour cells was mapped in three different regions of the tibia by multiphoton microscopy. Using Volocity 3D Image Analysis software we measured the distance between tumour cells and the nearest bone surface, and to other tumour cells. Competition studies were performed by partially occupying the niche by injecting $1x10^5$ DiD labelled MDA-MB-231-IV cells and repeating the injection seven days later with cells labelled with CM-DiI, allowing separate identification of both cell batches in the niche. The tumour cells preferentially homed to the trabecular area of the bones rather than to the growth plate ($p\leq0.005$). In animals receiving two batches of tumour cells, the number of cells homing to bone from the second batch was significantly lower compared with the cells from the first injection ($p\leq0.005$). Moreover, the preferential homing pattern changed, with tumour cells evenly located in different regions of the bone. Tumour cells were located significantly closer to the bone surface than to other tumour cells ($p<0.05$ and $p<0.01$), regardless of whether the niche was 'empty' or partially occupied. Our results show that the preferential pattern of tumour cell homing is modified when the niche is partially occupied, suggesting a degree of competition for space in the bone metastatic niche. (*In vivo* work covered by UK Home Office license PPL 40/3462.)
Disclosure: The authors declared no competing interests. MarieCurie ITN Training Network Agreement no.: 264817 (Bone-Net) and the CRUK program entitled 'Defining the Bone Metastasis Niche'.

CABS OC1.3

ERRalpha Regulates Prostate Cancer Cell Colonisation in Bone

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Eighty percent of patients dying from prostate carcinoma (PCa) have developed bone metastases that are incurable. Because we found the orphan nuclear receptor ERRa (oestrogen receptor related receptor alpha) expressed in bone metastases from PCa patients, we modulated its expression in PC3 cells. We showed that PC3 cells over-expressing wild-type ERRa (PC3-ERRa) stimulate rapidly osteolytic bone lesions in SCID mice (n=10) ((1,20±0,34* for osteolysis (mm^2) and 18,5±5,4** for skeletal tumor burden (TB/STV)(%)) compared with that observed with PC3-CT cells (0,49±0,22 (osteolysis) and 2±0,73 (TB/STV)) (Ethical approval DR2014-32). Surprisingly bone destruction was combined with new bone formation, as 70% of the metastatic limbs bearing PC3-ERRa cells had mixed lesions compared with CT-PC3 that are only osteolytic. Osteoclasts were directly affected *in vivo* and *in vitro* which was associated with the stimulation of pro-osteoclastic factors mRNA of Cox2, Runx2 and Cathepsin K by PC3-ERRa. Moreover, a statistical stimulation of bone formation in calvaria culture was observed when cells were co-cultured with PC3-ERRa. This was combined with the up-regulation of ET1, Wnt3a and Wnt5a that may explain the occurrence of bone formation *in vivo*. Interestingly, tumoural microenvironment was also affected by PC3-ERRa cells, as mouse periostin (POSTN), was over-expressed by the cancer-associated-fibroblasts *in vivo*. Moreover, we found that PC3-ERRa inhibits spheres formation, which was associated with a decrease of Nanog and Oct4 expression *in vivo*. Finally, we showed that elevated expression of ERRa mRNA in PCa (cohort of 60 patients) (Ethical approval CSTMT-042) is associated with high level of ET1, Cox2, POSTN and Wnt5a. In conclusion, our data provided for the first time evidence that ERRa can promote both osteolysis and osteosclerosis in animal models of PCa bone metastases. They also suggest its implication in the stromal niche via the POSTN/Wnt signalling and in the inhibition of the self-renewal capacity and pluripotency of tumour cells.

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CABS OC1.4

The Dark Side of Bone Anabolics? Intermittent PTH Modifies the Microenvironment to Increase Skeletal Breast Cancer Metastasis *In Vivo*

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Intermittent administration of parathyroid hormone (PTH) increases bone volume through positive effects on osteoblasts. However, it is not known whether expansion of osteoblastic cell populations also modifies breast tumour growth in bone. We have investigated the effects of PTH pre-treatment on bone and subsequent skeletal colonisation and growth of breast cancer cells *in vivo*. Twelve-week old female BALB/c nude mice (n=3-8/group) received PBS (control) or rhPTH 1-34 at 40/80ug/kg/day for 5 days +/- intracardiac injection of DiD-labelled MDA-MB-231-td-tomato-luc2 cells on day 5. Animal cohorts were sacrificed day 5, 7, 10, 15 (bone studies) or week 1, 2 or 9 (tumour studies). Bone was assessed by µCT, histology, bone histomorphometry and measurement of serum bone turnover markers. Tumour growth was monitored by *in vivo* imaging and bone homing investigated using two-photon microscopy. PTH treated animals had significantly increased numbers of osteoblasts compared with control (27.79 (40ug/kgPTH) and 24.07 (80ug/kgPTH) vs. 12.42(PBS) on day 5; $p<0.01$) and elevated serum P1NP levels (92.93:40ug/kgPTH and 89.31:80ug/kgPTH) vs. 30.87(PBS) day 7; $p<0.01$), whereas trabecular bone volume, osteoclast numbers and serum TRAP levels were unaffected. These effects were no longer detectable by day 10. Animals receiving tumour cell injections on day 5 of PTH treatment did not have higher number of colonising tumour cells in tibia/femur (90.6 in 80ug/kg PTH vs. 83.8 in PBS, $p>0.05$), and the number of tumours detected in the long bones was comparable (1.86:40ug/kgPTH) and 1.5:80ug/kgPTH) vs. 1.29(PBS), $p>0.05$). However, PTH caused increased tumour growth in skeletal sites not normally affected in this model, with higher number of tumours in sites outside the hind limbs (5.71:40ug/kgPTH and 5.25:80ug/kgPTH vs. 2.57(PBS); $p<0.0001$ and $p<0.001$). These results demonstrate that pre-treatment with PTH modifies the microenvironment, leading to increased breast tumour growth in a range of skeletal sites. (Covered by UK Home Office licence PPL40/3462.)

Disclosure: The authors declared no competing interests. This work was supported by a Cancer Research UK program grant “Defining the bone metastasis niche”.

CABS OC1.5

The Pain Mediator NGF is Induced by Multiple Myeloma *in vivo*, and Relieved by Therapeutic Activation of Adiponectin Signalling

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Multiple myeloma (MM) is a plasma cell neoplasm which causes osteolytic bone disease, and at diagnosis the most common symptom is bone pain. Adiponectin (Adpn) is a

myeloma-suppressive adipokine which negatively correlates with the major pain mediator Nerve Growth Factor (NGF) in osteoarthritis. We sought to determine whether NGF was increased in MM and the mechanism of NGF regulation within the MM-bone microenvironment. Mice were inoculated with 5TGM1-MM cells and tumour burden was associated with a significant increase in serum NGF ($p<0.01$). Immunohistochemistry revealed NGF expression in stromal cells within the growth plate, while CGRP+ nerves were detectable in the nearby periosteum. 5TGM1-MM or RPMI-8226 human MM cells did not express NGF, while cells found within bone (2T3 osteoblasts, ST2 bone marrow stromal cells (BMSCs), HS5 BMSCs and ATDC5 chondrocytic cells) expressed high NGF. Coculture of MM cells with BMSCs or osteoblasts significantly increased NGF expression in BMSCs or osteoblasts ($p<0.05$). MM-derived cytokines such as TNFα up-regulated NGF in 2T3 cells, ATDC5 chodrocytes and HS5 BMSCs ($p<0.05$). Adpn inhibited an LPS-induced increase in NGF in 2T3 osteoblasts and blockade of Adpn signalling by siRNA towards Adpn receptors AdipoR1 or AdipoR2 resulted in a significant 2-fold increase in NGF, which was further increased upon combination with TNFα. The Adpn-inducer L-4F was compared with bortezomib and melphalan *in vivo* in 5TGM1MM-bearing mice. All treatments gave a similar reduction in tumour burden (L-4F; 36%, bortezomib; 34%, melphalan; 36%, $p<0.01$), yet only L-4F induced a significant 2-fold reduction in serum NGF induction ($p<0.01$). Our results demonstrate that NGF is increased in MM *in vivo*, likely due to MM-induced up-regulation of stromal NGF. NGF is likely to be a cause of MM-induced bone pain, therefore Adpn-targeted therapies may provide an improvement over traditional approaches by reducing tumour burden while also acting to inhibit bone pain.

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CABS OC1.6

The Anti-Diabetic Drug Metformin Reduces Tumour Burden and Osteolytic Bone Disease in Multiple Myeloma *In Vivo*

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Multiple myeloma (MM) is a fatal haematological malignancy characterised by accumulation of malignant plasma cells in the bone marrow (BM), and severe lytic bone disease. Metformin is widely prescribed in diabetes, and is associated with improved outcomes in diabetic patients with MM, suggesting a potential anti-myeloma effect of metformin. The aim of the current study was to investigate the effect of metformin within the myeloma-bone microenvironment in vitro and *in vivo*. C57Bl/KaLwRij mice were inoculated with 5TGM1MM cells and treated with metformin either from time of tumour inoculation (continuous) or from time of established tumour (delayed). MM-bearing mice treated with metformin exhibited a decrease in myeloma-specific serum IgG2bk concentrations as compared to control (Control; 4.29 mg/ml ± 0.3 mg/ml, metformin-continuous; 1.51 mg/ml±0.6 mg/ml, $p<0.001$, metformin-delayed; 0.7mg/ml ± 0.7mg/ml, $p<0.001$). MicroCT analysis demonstrated a significant decrease in osteolytic lesion number in MM-bearing mice treated with metformin (Control; 26±3.6, metformin-continuous; 11.4±0.7, $p<0.001$, metformin-delayed; 9±1.5, $p<0.01$). Metformin induced a dose-dependent decrease in MM cell viability. MM cell lines exhibited a differential sensitivity to metformin; RPMI 8226 cells had highest basal metabolic activity and sensitivity to metformin. Metformin treatment of MM cells activated AMPK, decreased IGF-1 gene expression and induced apoptosis, detected by an increase in cleaved caspase-3 and PARP. Metformin had no effect on BM stromal cell (BMSC) viability. Direct contact of MM cells with BMSCs decreased the anti-MM effect of metformin. BMSC-conditioned media (CM) had a protective effect against the anti-MM effects of metformin at 24h that was lost by 72h. In contrast, BMSC CM protected against the anti-MM effects of the proteasome inhibitor bortezomib at all time points. Our studies demonstrate a strong anti-tumour effect of metformin in the MM-bone microenvironment, suggesting that metformin may be effective for the treatment of MM and the associated bone disease.

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CABS OC3.1

Targeting of Epithelial-to-Mesenchyme Transition by a Novel Small Molecule Inhibitor Attenuates Prostate and Breast Cancer Invasiveness and Bone Metastases

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Transformed epithelial cells can activate embryonic programmes of epithelial plasticity and switch from a sessile, epithelial phenotype to a motile, mesenchymal phenotype also referred to as epithelial-to-mesenchymal transition (EMT). EMT is associated with poor prognosis in patients with osteotropic cancers. E-cadherin (CDH1) is an essential homotypic cell adhesion molecule that is often down regulated during this process. EMT-like processes are increasingly linked to therapy resistance and metastasis-initiating cells, thus providing the rationale for the development of novel small-molecule inhibitors that a) block the acquisition of an invasive phenotype in osteotropic cancer cells via EMT or b) revert their invasive, mesenchymal phenotype into epithelial phenotype (MET) by upregulation of CDH1 expression. High throughput screening of >43,000 LMW compounds, followed by compound design & optimisation *in vitro* led to the identification ten candidate therapeutic compounds. These compounds displayed significant inhibitory effects on cancer cell invasion (>80%) and induced E-cadherin (re)expression, most likely through the interfere with the binding of transcriptional repressors to the *CDH1*

E-box elements. We identified a unique compound, OCD155, can effectively and dose-dependently block the acquisition of an invasive phenotype in osteotropic prostate and breast cancer cells (PC-3M-Pro4luc2 and MDA-MB-231/Bluc). When tested in our *in vivo* models of prostate and breast cancer bone metastasis, treatment of mice with OCD155 strongly and dose-dependently inhibited skeletal metastasis (number of metastases, tumour burden) according to preventive and curative protocols. At the dosages tested, no adverse effects of OCD155 were observed (body weight, liver toxicity parameters). To the best of our knowledge, our studies are the first to demonstrate the efficacy of new small molecule EMT inhibitor in the treatment of experimental skeletal metastasis.

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CABS OC3.2

BMP7 Inhibits Prostate Cancer Metastases by Depletion of Metastasis-Initiating Cancer Stem Cells

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Prostate cancer is the most common cancer in men, and bone is the preferred site for metastasis. Current treatment options for metastasised prostate cancer are not curative since conventional therapies (hormone, chemo-, and radiation therapy) seem relatively ineffective in targeting prostate cancer cells with stem/progenitor-like characteristics (CSCs). It is becoming increasing clear that the generation of CSCs may be linked to the acquisition of an invasive phenotype via epithelial-to-mesenchymal transition (EMT). Previously we found that bone morphogenetic protein (BMP)-7 inhibited EMT and bone metastasis formation but the effect of BMP7 on prostate CSCs has remained largely elusive. In this study, we show that BMP7 is the most potent inducer (180x) of BMP reporter signalling of all tested BMPs (BMP2, BMP4, BMP6, BMP2/7, BMP4/7). In clonogenic assays, BMP7 (2 nM) reduced the formation of holoclones (colonies enriched for CSCs) of PC-3 and PC-3M-Pro4 prostate cancer cells. While TGFβ pretreatment increased, BMP7 pretreatment reduced migration, and inhibited proliferation at later time points. Interestingly, when PC-3M-Pro4 cells were FACS-sorted for high aldehyde dehydrogenase (ALDH) enzymatic activity, BMP7 was shown to differentially inhibit clonogenic capability of the CSC subpopulation (ALDH-hi vs. ALDH-lo). *In vivo*, intravascular injection of red-fluorescent prostate cancer cells in zebrafish with a GFP+ vasculature -a model of dissemination and metastasis- led to rapid dissemination, extravasation and metastatic colonisation of distant sites. Strikingly, BMP7 pretreatment (2 nM) of PC-3M-Pro4/mCherry cells inhibited extravasation and reduced formation of distant metastases at 1 and 3 days post inoculation, respectively. Furthermore, BMP7 pretreatment increased the metastasis-free survival (62.5% vs 100% in vehicle) and reduced the number of distant metastases in a model of intracardiac injection of PC-3M-Pro4/Luciferase cells in nude mice allowing for real-time cell-tracking. In conclusion, we have shown that BMP7 targets the CSC subpopulation in human prostate cancer leading to impaired formation of distant metastasis.

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CABS OC4.1

Vectorisation of Hypoxia Activated Prodrugs to Chondrosarcoma Proteoglycans: Evaluation and Characterisation of Antitumoural Activity

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Chondrosarcoma, represents the second most frequent primary malignant bone tumour in adults after osteosarcoma. Because of its abundant chondrogenic extracellular matrix, its poor vascularisation and hypoxic microenvironment, chondrosarcoma is highly resistant to conventional chemo and radio-therapeutic treatments. Today, only effective treatment remains surgical resection. UMR990 Inserm/UdA laboratory develops a new innovative therapeutic targeting strategy which exploits the two characteristics of chondrosarcoma microenvironment: a chondrogenic extracellular matrix (ECM) and a hypoxic tissue. Based on the affinity of the quaternary ammonium (QA) moiety for proteoglycans, we developed a strategy that uses the quaternary ammonium function to selectively address hypoxia activated prodrugs to the ECM of chondrosarcoma, that exhibit a high fixed charged density (FCD). We propose thus, to vectorise, with QA as vectors to PGs of chondrosarcoma, cyclophosphamide derivative hypoxia activated prodrugs with nitroimidazole or nitrofurane cleavable entity. These compounds were studied *in vitro* and *in vivo* comparatively to their non-vectorised equivalents and to a vectorised but non cleavable equivalent. The *in vitro* results, on the HEMC-SS human chondrosarcoma cell line, show that QA derivatives of nitroimidazole prodrug exhibited the best hypoxia versus normoxia differential cytotoxic activity (4.5 times more apoptotic cells in hypoxia than in normoxia). *In vivo*, on an HEMC-SS xenograft SCID mice model, this molecule causes a significant tumour growth inhibition of 62.1% as compared to only 8% for its non-vectorised equivalent. Interestingly, haematological side effects were less pronounced for the QA-prodrug respectively to the non-vectorised molecule. These highly promising results validate the approach of dual selectivity for chondrosarcoma treatment, especially for the nitroimidazole compound, by increasing its therapeutic index. This new innovative therapeutic strategy offers a real hope for treatment of cartilage cancer, relatively rare pathology, but particularly redoubtable.

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CABS OC4.2
Beta Haemoglobin (Hbb): a Novel Marker of Breast Cancer Progression

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Bone is the preferentially site of distant colonisation for breast carcinoma (BrCa). BrCa patients with metastases restricted to bone (BO) show a longer overall survival compared with BrCa patients developing bone and visceral metastases (BV). In a gene expression analysis, we found few genes whose expression was significantly different between the two groups of bone metastases. Among them, haemoglobin beta (HBB) was one of the most upregulated in BV vs. BO. Based on these data, we evaluated HBB expression in primary human BrCa, finding HBB in 34 out of 57 samples. Moreover, the percentage of HBB positive cancer cells was significantly higher in the invasive lesions than in the in situ counterpart (6-fold, $P<0.05$). Higher expression of HBB was also observed in ductal infiltrating carcinoma vs. the lobular invasive histotype (2.2-fold, $P<0.05$). Interestingly, a positive correlation ($P<0.05$) was observed between HBB and the Ki67 score. We next compared HBB expression between poorly aggressive (MCF7, HCC1954) and highly aggressive (MDA-MB-231) BrCa cells, finding a higher expression in the latter. HBB overexpression in MDA-MB-231 (MDA-HBB) and MCF7 (MCF7-HBB) cells increased migration and invasion ability compared with control (MDA and MCF7-empty), along with an increased expression of MMP9. Moreover, MDA-HBB and MCF7-HBB conditioned media induced *in vitro* tube formation (1.5-fold increase $P<0.05$). Consistently, the *in vivo* growth rate of orthotopically implanted MDA-HBB was higher compared with MDA-empty. Endpoint tumour weight was increased too (1.9 fold, $P=0.002$), while histology revealed less fibrosis in MDA-HBB and MCF7-HBB-derived tumours (0.4 fold, $P<0.001$) along with increased angiogenesis. Finally, local recurrence and visceral metastases were observed only in MDA-HBB implanted mice (incidence, 60%). Similar results were observed in MCF7-HBB orthotopically injected mice. Altogether, our findings demonstrate a positive correlation between HBB expression and BrCa aggressiveness, paving the way for the use of HBB as a BrCa progression marker.

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CABS OC4.3
FZD5 mediates the anti-proliferative, but not the pro-apoptotic effects of WNT5A on prostate cancer cells

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Wnt proteins and their cognate receptors play a significant role in malignant diseases, in particular in PCa. Previously, we could show that WNT5A inhibits PCa cell proliferation and induces apoptosis *in vitro*, leading to reduced PCa growth *in vivo*. However, the involved receptors remain unknown. Here, we determined which receptors mediate the WNT5A-induced effects on PCa cells. The expression profile of 12 different Wnt receptors was analysed in three human (PC3, C42B, MDA-PCa-2b) and two mouse (RM1, TRAMP-C2) PCa cell lines. Frizzled (FZD) 10 and FZD9 showed the lowest expression levels in the PCa cell lines, while FZD1; FZD6, and Ryk were highly expressed. To determine which receptors mediate the anti-proliferative and pro-apoptotic effects of WNT5A in PCa, we knocked down FZD5 and receptor tyrosin-like orphan receptor (ROR) 2 with specific siRNA in PC3 cells 24 h before the induction of WNT5A overexpression. After knock-down of ROR2, WNT5A was still able to suppress proliferation by 31%. However, FZD5 knock-down completely reversed the suppressive effect of WNT5A on proliferation. The knock-down of FZD5 and ROR2 itself did not change PCa cell proliferation. Interestingly, the increase of apoptosis after WNT5A overexpression could not been reversed by neither knock-down of FZD5 or ROR2, suggesting another receptor involved in this process. Of note, knock-down of FZD5 even further increased apoptosis after WNT5A overexpression. A cDNA array containing samples from 9 healthy and 39 patients with prostate cancer was evaluated for WNT5A, FZD5 and ROR2 expression. WNT5A, FZD5, and ROR2 mRNA expression was significantly higher in the prostate cancer samples compared with healthy controls ($p<0.001$). However, only FZD5 expression correlated highly positively with WNT5A expression ($r^2=0.8801$, $p<0.001$). These data suggest that FZD5, but not ROR2, mediates the anti-proliferative effects of WNT5A on prostate cancer cells.

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ORAL POSTERS - CABS

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CABS OP1.1 (P133)

Cripto/GRP78 Signalling in Dissemination and Metastasis of Human Osteotropic Prostate Cancer

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Prostate cancer is the most prevalent cancer in men and metastatic spread to bone is detected in up to 80% of the patients with advanced disease. Cell surface oncoproteins are attractive therapeutic targets, readily accessible to antibodies and other membrane impermeable protein/peptide-based anticancer agents. Cripto is a GPI-anchored cell surface/secreted oncoprotein that plays important roles in embryogenesis, stem cell maintenance and tumour progression. GRP78 is a HSP70 family member that binds Cripto at the cell surface. We recently found that Cripto and GRP78 are both highly expressed in human castration resistant prostate cancer (PCa), but not in androgen-dependent tumours. We investigated if Cripto/GRP78 signalling promotes the aggressive, stem cell-like PCa phenotype associated with castration resistance and bone metastasis. To mimic the endosteal metastatic niche, highly metastatic human PC-3M-Pro4luc2 prostate cancer cells were cultured with primary human osteoblasts. We found that the presence of human osteoblasts reduces the proliferation of PC-3M-Pro4luc2 cells and results in induction of the E-Cadherin repressor ZEB1, causing the PCa cells to acquire a more mesenchymal, invasive phenotype as reflected by their reduced E-Cadherin/Vimentin ratio. Co-culture of PC-3M-Pro4luc2 cells with osteoblasts also greatly increased the ALDHhigh/ALDHlow ratio indicating an increase in the size of the metastatic stem/progenitor cell population. This increase in the ALDHhigh subpopulation corresponded to enhanced Cripto and GRP78 expression and stable knockdown of Cripto or GRP78 reduced PC-3M-Pro4luc2 proliferation and clonogenicity, and decreased the size of the metastasis-initiating ALDHhigh subpopulation. Finally, we used zebrafish as a model system for measuring tumour cell dissemination and metastasis and found that Cripto knockdown in PC-3M-Pro-4luc2 cells led to a significant reduction in metastatic tumour burden. In conclusion, our findings point to a potential role for Cripto and GRP78 in driving metastatic, therapy-resistant phenotype and suggest that targeting the Cripto/GRP78 pathway may have significant therapeutic potential.

CABS OP1.2 (P132)

Contribution of Osteocytes to Cancer-Associated Bone Pain via Connexin43-Mediated Communications with Sensory Neurons Under the Acidic Microenvironment in Bone Metastasis

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Bone pain is one of the most prevalent and devastating complications of cancer in bone. The pathophysiology of cancer-associated bone pain (CABP) is poorly understood but likely involves complex interactions among the cancer cells, peripheral sensory nerves and bone cells. Recent studies reported that the calcitonin gene-related peptide-positive (CGRP+) sensory neurons densely innervate mineralised bone, in which numerous osteocytes are present, leading us to hypothesise that osteocytes interact with these CGRP+ sensory neurons to evoke CABP. We tested this hypothesis using an animal model in which inoculation of the JJN3 human multiple myeloma (MM) cells into tibiae induced progressive CABP. We found that JJN3 MM-colonised bone was acidic and that blockade of the acidification by the proton pump inhibitor bafilomycin A1 significantly reduced the CABP. Immunohistochemical examination demonstrated that osteocytes localised in the close proximity of CGRP+ primary afferent sensory neurons in mineralised bone. Co-culture of MLO-A5 osteocytic cells and F11 sensory neuronal cells showed that MLO-A5 cells transferred the permeable living dye calcein to F11 cells by extending dendritic processes to contact the neurites of F11 cells. The general gap junction inhibitor 18β-GA and the selective connexin43 (Cx43) blocker GAP27 and silencing Cx43 in MLO-A5 cells by shRNA all decreased the dye transfer, suggesting that the Cx43 gap junction mediates the osteocyte-sensory neuron communication. Determination of neuronal excitation by Ca^{2+} influx imaging assay showed that the acidic medium excited F11 sensory neuronal cells. Importantly, acid-induced excitation of F11 cells was enhanced in the presence of MLO-A5 osteocytic cells and GAP27 and silencing Cx43 abolished acid-induced F11 excitation in the co-cultures. In conclusion, our results suggest that osteocytes contribute to the pathophysiology of CABP via Cx43-mediated communications with sensory neurons innervating bone. These communications may be a novel therapeutic target in the management of CABP.

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CABS OP1.3 (P136)

Endothelin-1, a Gene Regulated by TMPRSS2:ERG Fusion Proteins in Prostate Cancer Bone Metastases

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Bone metastases are frequent and severe complications of prostate cancer (PCa). Recently, the *TMPRSS2:ERG* gene fusion, which results in the aberrant androgen-dependent expression of the ERG transcription factor, has been shown to be the most common gene rearrangement in PCa. This study investigates a potential role of the gene fusion in the development and phenotype of PCa bone metastases. We previously established cell clones from a PCa cell line (PC3c), over-expressing different levels of TMPRSS2:ERG. *In vivo* analysis of bone lesions induced by intra-tibial injections of PC3c-TMPRSS2:ERG clones in mice (ethical approval DR2014-32) showed an increase of osteoblastic phenotype compared with control cells. Furthermore, a transcriptomic study of these clones showed a change of expression in many genes, including *endothelin-1* (*ET-1*). Since ET-1 is known to be involved in osteoblast proliferation and in osteoblastic metastasis formation in PCa, we therefore investigated the transcriptional regulation of *ET-1* by fusion proteins. *In vitro*, we have shown that this gene was overexpressed in PC3c-TMPRSS2:ERG clones, depending on ERG expression levels, and was inhibited by ERG silencing. *In silico* analysis of the promoter of *ET-1* revealed the presence of several potential binding sites of ERG. Chromatin immunoprecipitation experiments demonstrated a direct binding to one of them. Moreover, using a cohort of human carcinoma prostate samples (ethical approval CSTMT-042), we were able to establish a correlation between the expression of *ET-1* and the expression of the fusion gene *TMPRSS2:ERG*, reinforcing the link between *ET-1* and the fusion. Taken together, these results strongly suggest that the *TMPRSS2:ERG* gene fusion contributes to the osteoblastic phenotype of PCa bone metastases and that ET-1 is a crucial target gene regulated by the transcription factor ERG.

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CABS OP3.1 (P131)

The Inhibition of c-MET Reduces Bone Metastases Induced by Renal Cancer Stem Cells

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Renal cancer patients often develop particularly destructive bone metastases. In solid tumours, cancer stem cells (CSCs) directly promote bone metastasis, thus therapeutic strategies to block the interaction between CSCs and bone microenvironment are currently under investigation. Since c-MET mediates the interaction between cancer cells and mesenchymal cells of the bone microenvironment, we hypothesised that targeting c-MET will lead to bone metastases inhibition. Renal CD105+ CSCs isolated from human cancer patients were injected in NOD/SCID mice, previously implanted with a small fragment of human bone. After the injection of CSCs, mice were daily treated or not with a c-MET inhibitor (JNJ) for 90 days, then sacrificed. Importantly renal CSCs colonised human implanted bone but not mice bone, leading to a specie-specificity of those cells to metastasize human bone. We then found that the JNJ treatment inhibited metastatisation at bone implant site. We studied the effect of JNJ on osteoclasts (OCs) and osteoblasts (OBs) of the bone implant by histomorphometry, showing that CSCs induced an activation of OCs corresponding to an increase erosion surface, whereas the OB activity diminished with a reduction of the osteoid thickness. The treatment with JNJ restored the normal activity of OCs and OBs, comparable with the control mice. Then we investigated the effect of JNJ on *in vitro* cultures of human OCs and OBs, to avoid the bone microenvironment interference. JNJ reduced the number of TRAP+ OCs, whereas it did not significantly affect the number of BAP+ OBs. Furthermore, we analysed mice sera by a multianalyte detection system, showing that IL-11 and CCL20 levels are higher in mice untreated with JNJ than in treated ones, suggesting a role of these molecules in the CSC bone metastatic process. Our results highlight the ability of this c-MET inhibitor to abrogate the bone metastastis formation induced by renal CSCs.

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CABS OP3.2 (P134)

Calpain-6 Expression Identifies a Stem Cell Population in Osteosarcoma

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Identification of cancer stem cells in carcinomas has proved to be useful to understand cancer progression and for prognostic purpose. However, the properties of osteosarcoma stem cells remain challenging and controversial, mainly due to the lack of functional markers to study such cells *in vivo*. Previously, we identified calpain-6 as a protective factor involved in chemoresistance process of osteosarcoma. To investigate the mechanisms controlling its expression we characterised 7285 bases of the regulatory sequence in calpain-6 gene. This sequence comprises an active promoter and multiple functional binding sites for embryonic stem cell factors such as Oct4, Nanog and Sox2 as shown by rapid cDNA end amplification and chromatin precipitation. Silencing Oct4, Nanog or Sox2 was sufficient to reduce basal and hypoxia dependent up-regulation of calpain-6 expression and the activity of the regulatory sequence cloned upstream the luciferase gene reporter. This indicates that calpain-6 is controlled by the stem cell transcription factors. To further document a possible relationship between Calpain-6 and a stem cell phenotype, we used GFP as gene reporter, to identify the cells in which the calpain-6 promoter was activated. Culturing osteosarcoma cell lines on non-adherent plastic and in minimal medium allowed obtaining spheroids that were previously shown to be enriched in tumourigenic stem-like cells. Calpain-6 protein was up regulated in spheres obtained from human 143B cells as compared with adherent cultures. Moreover, GFP positive cells sorted from adherent cultures have higher capacities to form spheroids than GFP negative cells. These GFP positive cells also expressed higher RNA levels of the embryonic stem cell markers, c-MYC and ABCB1. Five weeks after injection into the tibia of BALB/c mice, GFP-positive K7M2 cells formed tumours that produced a high luminescent signal as compared with tumours formed from GFP-negative cells that are largely necrotic. In *in vitro* scratch tests, migrating cells were found to express high levels of calpain-6 and GFP-positive cells displayed higher capacities for migration than negative ones, whereas, calpain-6 shRNA reduced these capacities. Finally, intra bone injection of GFP-positive cells resulted in more metastatic lesions in lungs than negative cells indicating that calpain-6 is involved in metastatic process. Altogether, our data show that calpain-6 expression is regulated by transcription factors that control multipotency and renewal of embryonic stem cells. Calpain-6 identifies an osteosarcoma cell population that express stem markers and with higher chemoresistance, migration capacities and tumourigenicity. The reporter system driven by calpain-6 regulatory sequence may therefore represent a powerful tool to further study stem cells in osteosarcoma.

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CABS OP3.3 (P137)

EPCR Promotes a Tumourogenic and Metastatic Phenotype in Breast Cancer

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Endothelial protein C receptor (EPCR) is a transmembrane receptor widely expressed in endothelial cells where it exerts cytoprotective and anticoagulant activities. We have shown that it is also expressed in lung tumour cells where it promotes tumour cell survival and increases osseous prometastatic activity. However, to date the contribution of EPCR to tumourogenesis and skeletal metastasis in breast cancer remains ill defined. Lentiviral shRNA-mediated EPCR silenced (shEPCR) cells in the MDA-MB-231 derived 1833 breast cancer cell line showed unaltered growth kinetics in basal or apoptotic-induced *in vitro* conditions. However, EPCR silencing reduced tumour growth in an orthotopic model of mammary fat pad injection. Interestingly, intracardiac inoculation of shEPCR cells led to a substantial reduction in skeletal metastatic burden, assessed by bioluminescence imaging, and osteolytic lesions, evaluated by micro-X-Ray imaging, micro-CT scans and histological analysis. This effect was associated with a decreased skeletal tumour growth observed after intratibial inoculation of shEPCR cells as compared with control cells. Furthermore, after intra-tail injection of the murine breast cancer cell line ANV5, we found a dramatic decrease in lung metastasis in animals injected with shEPCR cells as compared with control mice, despite the similar growth kinetics of the cell lines *in vitro*. *In vivo* transcriptomic analysis identified several relevant signalling pathways differentially altered in shEPCR and control tumours. To explore the clinical relevance of these findings we carried out global expression analysis in a cohort of 286 patients. Patients with high EPCR expression levels had shorter relapse-free survival times as compared with patients with low EPCR expression levels. These data indicate that EPCR confers an *in vivo* protumourogenic and prometastatic phenotype to bone and lung. Monitoring EPCR could represent a clinically relevant factor in breast cancer and a potential therapeutic target.

Disclosure: The authors declared no competing interests.

CABS OP3.4 (P130)

Bone Cells Control Myeloma Cell Dormancy and Activation in the Skeleton

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Multiple myeloma predominantly grows in bone, causing extensive destruction. Despite targeted therapies, relapse is common and the disease remains incurable. To develop more effective treatments we need an improved understanding of

myeloma cell engraftment, dormancy and reactivation in the skeleton. We hypothesise that myeloma cells engage in an endosteal niche in which they reside in a dormant state, resist chemotherapy and can be reactivated through changes in the local environment, contributing to disease relapse. To address this, we have developed *intravital* imaging to study tumor cell colonization of the endosteal niche and tumour cell dormancy and reactivation. 5TGM1eGFP murine myeloma cells were labelled with the membrane dye DiD. *In vitro*, DiD label is lost through division, distinguishing dormant (DiD^{+ve}/GFP^{+ve}) from proliferating cells (DiD^{Neg}/GFP^{+ve}). Myeloma cells were injected into C57BLKalwRij mice and treated with melphalan (3 times/week, 5mg/kg days 14-28), sRANKL (daily days 4-6) or vehicle. Using *intravital* microscopy, individual dormant DiD^{+ve}/GFP^{+ve} cells were visualised at 7, 14, 21, or 28 days post injection and located in endosteal niches. By day 14, a limited number of myeloma cells were activated to form growing DiD^{Neg}/GFP^{+ve} colonies which were localised distant from bone surfaces. Melphalan treatment reduced tumour burden (<97%), however dormant DiD^{+ve}/GFP^{+ve} tumour cells remained. Following removal of melphalan treatment, tumor burden increased and DiD^{+ve}/GFP^{+ve} cells reduced, indicating that re-activation had occurred. Lastly, sRANKL stimulation of osteoclast activity reduced dormant (DiD^{+ve}/GFP^{+ve}) cells, suggesting osteoclast driven increased reactivation of dormant tumour cells. Taken together, these data show that dormant tumour cells, which reside in endosteal niches, resist chemotherapy and are available to repopulate the tumour. Importantly, we demonstrate that increased osteoclast remodelling of the endosteal niche reactivates tumour cells in the skeleton. These data provide insights into the fate of dormant cells, mechanisms behind drug resistance and identifies new mechanisms for disease relapse.

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CABS OP3.5 (P138)

Hif Signalling in Skeletal Progenitors Promotes Breast Cancer Growth and Metastasis Through Systemic Production of CXCL12

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High bone mineral density (BMD) has long been associated with increased risk of breast cancer. Conversely, low bone mass has been correlated with lower risk of breast cancer. Although BMD was initially thought to reflect a cumulative exposure to oestrogens, recent clinical trials demonstrated that high bone mass correlates with elevated breast cancer incidence independently of reproductive correlates, endogenous and exogenous exposure to oestrogen. However, the biological mechanism linking bone mass and the risk of breast cancer is unknown. Our objective was to investigate the role of the osteoblastic lineage in breast cancer, using transgenic mice presenting increased or decreased bone mass (all animal protocols were approved by an animal ethics committee). Here we show that osteoprogenitor cells, targeted by Osterix driven Cre-recombinase, exert a systemic control of breast cancer growth and metastasis. Deletion of the tumour suppressor gene von Hippel Lindau (Vhlh) specifically in mouse osteoprogenitors ($Osx/Vhlh^{fl/fl}$), which results in increased protein level of the Hypoxia-Inducible Factor-1alpha (Hif-1alpha) in these cells, led to increased bone mass, and increased mammary tumour growth and metastasis. Conversely, deletion of Hif-1alpha in osteoprogenitors ($Osx/Hif\text{-}1alpha^{fl/fl}$) decreased bone mass, and dampened mammary tumour growth and metastasis. We found that changes in the bone microenvironment are associated with changes in the plasmatic levels of the chemokine C-X-C motif ligand 12 (CXCL12). Pharmacological inhibition of the CXCL12-CXCR4 pathway abolished increased primary tumour growth and dissemination in $Osx/Vhlh^{fl/fl}$ mice. Therefore, skeletal dysfunction alters tumorigenesis beyond the bone microenvironment. Our results provide a mechanistic explanation as for why high bone mass is linked to increased risk of breast cancer, and support the notion that the skeleton is an important organ of the tumour macroenvironment. They also indicate that drugs affecting bone homeostasis may have important consequences in breast cancer.

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CABS OP4.1 (P129)

miR-25 and miR-21 Regulate Prostate Cancer Invasiveness by Attenuation of Notch-TGF-β Crosstalk and Self-Renewal Markers

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Altered microRNA (miR) expression is associated with tumour formation and progression of various solid cancers. A major challenge in miR profiling of bulk tumours is represented by the heterogeneity of the subpopulations of cells that constitute the organ and tumour tissue. We analysed the expression of miRs in a subpopulation of bone metastasis-initiating stem/progenitor-like cells in human prostate cancer (PCSC) and compared with more differentiated cancer cells. In PC-3M-Pro4Luc2 and C4-2B prostate cancer cell lines and clinical prostate cancer specimens we identified that miR-25 and miR-21 expression in PCSCs was low/absent and steadily increased during their differentiation into cells with a luminal epithelial phenotype. Functional studies revealed that overexpression of miR-25 in prostate cancer cell lines and selected subpopulation of highly metastatic/tumourigenic cells ($ALDH^{high}$) strongly affected the invasive cytoskeleton reducing migration *in vitro*, while overexpression of miR-21 reduced the size of $ALDH^{high}$ subpopulation. Additionally, miR-25 overexpression dramatically decreased the expression of Notch1 and Jagged1, critically involved in aetiology of skeletal metastasis, together with other Notch downstream targets in prostate cancer cells, while miR-21 downregulated self-renewal markers.

Moreover, we found that miR-25 decreased TGF-β signaling in human prostate cancer cells and that miR-25 overexpression blocks the induction of Jagged1 driven by TGF-β. In line with these observations, we further demonstrate that miR-25 can act as a tumour suppressor in highly metastatic PCSCs by direct functional interaction with the 3´UTR of pro-invasive α_6 and α_v- integrins. Finally, we show here for the first time, that miR-25 can reduce metastasis by blocking the extravasation of human prostate cancer cells *in vivo.* Taken together, our observations suggest that miR-21 and miR-25 are key regulators of invasiveness in human prostate cancer through direct interactions with α_v- and α_6 integrins & Notch1 expression.
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CABS OP4.2 (P128)

Targeting Runx2 By Mir-135 and Mir-203 Impairs Breast Cancer Metastasis and Progression of Osteolytic Bone Disease

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Progression of breast cancer to metastatic bone disease is associated with an aberrantly elevated expression of Runx2, which promotes disease progression through transcriptional activation of genes involved in metastasis. Inhibition of Runx2 in metastatic breast cancer cells prevents metastatic bone disease, thus providing a basis for Runx2 as a potential therapeutic target. Since transcription factors are challenging to target for therapeutic intervention, our goal was to evaluate the potential clinical use of Runx2-targeting microRNAs (miRNAs) to reduce tumour growth and bone metastatic burden. Expression analysis of a panel of miRNAs regulating Runx2 revealed a reciprocal relationship between the abundance of Runx2 protein and two miRNAs, miR-135 and miR-203. These miRNAs are highly expressed in normal breast epithelial cells where Runx2 is not detected, and conversely are absent in metastatic breast cancer cell lines and importantly, in tissue biopsies that express Runx2. Reconstituting metastatic MDA-MB-231-luc cells with miR-135 and miR-203 reduced the abundance of Runx2 and the expression of the metastasis-promoting Runx2 target genes. Additionally, tumour cell viability was decreased and migration suppressed *in vitro*. *In vivo* implantation of MDA-MB-231-luc cells reconstituted with miR-135 or miR-203 into the mammary gland, followed by additional intratumoural administration of the synthetic miRNAs reduced tumour growth and importantly, spontaneous metastasis to bone. Furthermore, intratibial injection of these cells impaired tumour growth in the bone environment, inhibited bone resorption and secondary metastasis to lung. Importantly, reconstitution of Runx2 in MDA-MB-231-*luc* cells delivered with miR-135 and miR-203 reversed the inhibitory effect of the miRNAs on tumour growth and metastasis. We conclude that aberrant expression of Runx2 in aggressive tumour cells is related to the loss of specific Runx2-targeting miRNAs and that a clinically relevant replacement strategy by delivery of synthetic miRNAs is a viable therapeutic approach to target transcription factors for the prevention of metastatic bone disease.
Disclosure: The authors declared no competing interests.

CABS OP4.3 (P139)

Radium-223 Dichloride Exhibits Dual Mode-of-Action Inhibiting both Tumour and Tumour-Induced Bone Growth in Two Osteoblastic Prostate Cancer Models

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Radium-223 dichloride, an alpha particle-emitting calcium-mimetic, improves overall survival in prostate cancer patients with symptomatic bone metastases. Here, we define radium-223 mode-of-action and efficacy in two clinically relevant prostate cancer xenograft models. Human LNCaP or patient-derived LuCaP 58 prostate cancer cells were inoculated intratibially and mice were stratified into treatment groups based on lesion grade and/or serum PSA levels. Radium-223 (300 kBq/kg) or vehicle was administered twice at 4-week intervals. X-rays and serum samples were obtained biweekly. Bone samples were collected for γ-counter measurements, micro-CT, autoradiography and histology. Radium-223 inhibited tumour-induced osteoblastic reaction as indicated by reduced bone volume and surface area in both prostate cancer models. Additionally, radium-223 suppressed metabolic activity in bone as evidenced by decreased osteoblast and osteoclast numbers and reduced PINP levels. Radium-223 treatment also resulted in lower PSA levels as early as two weeks post first dosing, indicating constrained tumour growth. This phenomenon was further supported by reduced tumour area in tibia in both models and an overall increase in necrotic tumour area in the LuCaP 58 model. Moreover, DNA double-strand breaks were increased in cancer cells 24 hours post radium-223 administration in the LuCaP 58 model providing further evidence of anti-tumour effects. Autoradiography confirmed radium-223 deposition in the intratumoural bone matrix in conjunction with osteoblasts. We demonstrate that radium-223 dichloride is successfully incorporated into the intratumoural bone matrix and inhibits tumour growth in both cell line- and patient-derived osteoblastic prostate cancer models. Importantly, given the α-particle range of 50-80 µm, potent radiation effects on the immediate tumour microenvironment are expected with minimal or no effects on the more distant bone marrow. Taken together, radium-223 therapy exhibits

a dual mode-of-action that impacts on tumour growth and tumour-induced bone reaction, both important players in the destructive vicious cycle of osteoblastic bone metastasis in prostate cancer.
Disclosure: MIS, JPR, and JMH are stockholders of Pharmatest Services; EA and SK are consultants of Pharmatest Services; DM, KZ and AS are stockholders of Bayer Pharma AG.

CABS OP4.4 (P135)
Salinomycin Treatment Inhibits Prostate Cancer Growth *In Vitro*, *In Vivo* and in Near-Patient *Ex Vivo* Models

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Prostate cancer (PCa) is the most common cancer in men, and up to 70-80% of patients with advanced disease present with bone metastases. Current treatment options for metastasised PCa are not curative since hormone, chemo-, and radiation-therapy are relatively ineffective in targeting PCa cells with stem/progenitor-like characteristics (CSCs). Salinomycin, an antibiotic used in poultry, was previously identified in a high through-put screen to target breast CSCs 100x more effectively than paclitaxel. In this study we investigated the anti-tumour effects of salinomycin in human PCa cells *in vitro*, *in vivo* and *ex vivo*. Salinomycin dose-dependently inhibited the proliferation of various human PCa cells (PC3, PC-3M-Pro4, DU145, C4-2B, PC339, PC346C). Interestingly, after establishing docetaxel-resistant cells by serial passaging *in vivo* (PC339-DOC), salinomycin differentially affected docetaxel-resistant cells (vs. parental). Salinomycin induced apoptosis as determined by flow cytometry (Ann/PI) and immunohistochemistry (caspase-3), reduced Notch-signalling (RBPkJ/Luc reporter assay) and inhibited migration of PC-3M-Pro4 cells. When PC-3M-Pro4 cells were FACS-sorted for high aldehyde dehydrogenase (ALDH) enzymatic activity, salinomycin inhibited the clonogenic and sphere-forming capacity of both CSC and non-CSCs equally well. Salinomycin pretreatment of PC-3M-Pro4/mCherry completely blocked extravasation and metastatic colonisation in a zebrafish model with a GFP+ vasculature in which cells were intravascularly injected. Salinomycin pretreatment of PC-3M-Pro4/luc2 cells also reduced the formation of distant metastases in a bone metastasis model of intracardiac injection of cancer cells in nude mice. *Ex vivo*, salinomycin treatment for 7 days (vs. vehicle treated) strongly reduced the number of PCa cells in a novel 'near-patient' model of culturing prostate tumor slices from transurethral resection of prostate cancer tissue (TURP) and bone metastases. In conclusion, salinomycin is effective in inhibiting PCa growth *in vitro*, *in vivo* and in near-patients *ex vivo* models. Therefore, salinomycin may be a promising novel therapeutic approach for the treatment of advanced, bone metastatic PCa.
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OP1 (P16)

HR-pQCT and DXA Changes in Bone Density and Microarchitecture Over Two Years in Young Adults

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Timing of peak bone microarchitecture parameters measured with high-resolution peripheral quantitative computed tomography (HR-pQCT) may differ from dual x-ray absorptiometry (DXA) due to resolution or skeletal site differences. We aimed to assess differences in timing of peak values for microarchitecture and bone density using HR-pQCT and DXA. Females (n=42, 21.5 yrs) and males (n=33, 21.6 yrs) from the Calgary youth cohort of the Canadian Multicentre Osteoporosis Study (CaMos) participated in a 2-year follow-up study. DXA (Hologic, USA) scans of the left hip provided areal bone mineral density (aBMD) of femoral neck (FN) and total hip (TH). Non-dominant radius and left tibia were scanned using HR-pQCT (Scanco Medical, Switzerland). To compare repeat scans, automated 3D image registration was conducted (IPL software). Total volumetric BMD (Tt.BMD), cortical BMD (Ct.BMD), trabecular BMD (Tb.BMD) and cortical porosity (Ct.Po) were assessed. Repeated measures ANOVA determined age-related bone change. DXA-derived aBMD decreased at the hip for females and males by 0.5-1.0% per year ($p<0.01$). At the radius, volumetric BMD increased by 0.6-1.0% per year for males and females ($p<0.01$). There were no significant changes in Ct.Po at the radius ($p>0.05$). At the tibia, there were no significant changes in volumetric BMD; however, Ct.Po increased 7% for females and 10% per year for males ($p<0.01$). Our findings are consistent with known DXA peaks occurring before 20 years at the hip. An increase in HR-pQCT-derived BMD parameters at the radius, suggests peak density at the radius occurs at an age >22 years. At the tibia, all HR-pQCT-derived BMD parameters remained stable, suggesting peak density may occur before 22 years. Like DXA, timing of peak HR-pQCT values differ according to skeletal site (radius vs. tibia). Cohorts used for HR-pQCT normative data should include recruitment of subjects <22 years of age to capture peak measurements for all sites.

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OP2 (P48)

A New Method for 3D-QCT of the Distal Forearm Using Clinical Whole-Body CT Scanners

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Introduction: For peripheral QCT usually dedicated scanners like the XtremeCT (ScancoMedical AG, Switzerland) are used. However, scan times are long and only small volumes or single slices can be acquired. In contrast, with widely available clinical whole-body CT scanners, 10 to 20cm long scans of both distal forearms can be acquired in seconds. We developed a 3D analysis method using 3D segmentation and an automatic placement of analysis volumes of interest (VOIs) for determining bone mineral density (BMD), content (BMC) and cortical thickness.
Methods: 23 datasets of both distal forearms of young professional male climbers (n=11) and age and BMI matched healthy controls (n=12) were acquired on a SIEMENS VolumeZoom (80kV, 122mAs, 24cm FOV, 15cm scan, 1.0mm slice thickness, kernel B60s, Siemens Osteo Phantom for BMD calibration). Endosteal and periosteal surfaces of radius and ulna were segmented using a multi-step local adaptive thresholding procedure. Four anatomically adapted VOIs (ultra-distal, distal, mid, proximal) were automatically defined in the radius.
Results: Integral, cortical and trabecular BMD and BMC and cortical thickness were measured in each VOI (results are denoted as: climbers, controls, p-value). Young adult climbers showed significantly increased integral and cortical BMD (ultra-distal: 762±50mg/cm³, 653±48mg/cm³, $p<0.001$; distal: 1074±35mg/cm³, 1018±59mg/cm³, $p<0.05$) and BMC as well as cortical thickness (ultra-distal: 0.97±0.08mm, 0.87±0.06mm, $p<0.001$; distal: 1.2±0.1mm, 1.1±0.1mm, $p<0.05$) in both distal VOIs. Trabecular BMD (170±18mg/cm³, 142±36 mg/cm³, $p<0.05$) and BMC was significantly higher in the ultra-distal VOI. Results for mid and proximal VOIs were not significantly different.
Conclusion: A new 3D-QCT analysis program for the distal forearm was developed specifically exploiting advantages of whole-body CT scanners. It can be used to determine BMD, BMC and cortical thickness at any position along the radius included in the scan. The study showed that climbing predominantly affects bone parameters in the most distal regions of the forearm.

OP3 (P391)

Increased Cortical Porosity in Women and Men with Diabetes: the Framingham Osteoporosis Study

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Although type 2 diabetes mellitus (DM) patients have normal or increased BMD as determined by DXA, risk of fracture is greater in DM than non-DM. This paradox has led to investigation of deficits in skeletal microarchitecture that may be responsible for increased fracture risk in DM. We conducted a community-based study of women and men to compare bone microarchitecture by DM status. Participants included 627 members (367 women, 260 men) of the Framingham Osteoporosis Study, mean age 65 yrs (range, 45-84). We defined DM as fasting glucose (FG) $\geq$126 mg/dl or use of DM medication and pre-DM as FG=100-125 mg/dl. Bone microarchitecture and density of the tibia and radius were measured by HR-pQCT (XtremeCT, SCANCO). Linear regression was used to calculate means (± SEs) for HR-pQCT bone indices according to DM status, adjusted for age, sex, and weight. 71 (40 men, 31 women) cohort members had DM (11%). At the tibia, persons with DM had significantly higher cortical porosity (11.17% ± 0.38 vs. 10.03% ± 0.13, p<0.01) and lower cortical vBMD (796.74 ± 8.02 vs. 814.00 ± 2.76, p=0.04) compared with non-DM. In contrast, trabecular vBMD and trabecular number were higher in DM, although differences were not statistically significant. Further, cortical porosity in the tibia was highest in DM (11.19% ± 0.38), intermediate in pre-DM (10.10% ± 0.19), and lowest in the non-DM group (9.95% ± 0.18); trend, p=0.02. HR-pQCT measures at the radius were not associated with DM. Results were similar when stratified by sex. In this community-based study, we found that women and men with DM had deficits in cortical bone at the tibia. To reduce the burden of skeletal fragility in DM, it will be important to determine whether deficits in cortical bone explain increased fracture risk observed in older adults with DM.

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OP4 (P218)

Serum Sclerostin is Associated with Impaired Insulin-Induced Whole-Body Glucose Uptake in Obesity

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Background: Disturbed Wnt signalling has been implicated in numerous diseases, including type 2 diabetes and the metabolic syndrome. Wnt and insulin signalling pathways exhibit cross-talk at multiple levels. Wnt proteins enhance phosphorylation of insulin signaling molecules in skeletal muscle cells. Sclerostin is a bone-derived circulating inhibitor of the Wnt signalling pathway and is increased in obesity. Obesity is also characterised by defects in insulin signalling in muscle cells resulting in peripheral insulin resistance. It is hitherto not reported whether circulating Wnt signalling inhibitors are related to impaired insulin-induced effects on glucose uptake in lean and/or obese individuals. To examine whether serum sclerostin is associated with insulin-induced whole-body glucose uptake and whether this association differs between obese and lean individuals.

Methods: Whole-body glucose uptake (WBGU) was assessed by a hyperinsulinaemic euglycaemic clamp and expressed per lean body mass in lean and obese healthy women (BMI$\leq$25 vs. BMI$\geq$30 kg/cm^2). Serum sclerostin was measured using the MesoScale Discovery chemiluminescence assay.

Results: Twenty-one lean and 21 obese women were included (mean±SD; age 37±11 years, BMI 22.0±2.0 kg/cm^2 and n=21; age 39±12 years; BMI 34.8±4.9 kg/cm^2, respectively). Serum sclerostin was higher in obese as compared with lean women (123±33 vs. 93±33 ng/L, p=0.006). Insulin infusion did not affect serum sclerostin. Due to interaction for obesity in the relationship between serum sclerostin and WBGU (p=0.014), further analyses were stratified for obesity. In obese but not lean women serum sclerostin was related to WBGU (-0.089±0.028 mg•kg^{-1}•min^{-1}, p=0.005) after adjustment for age. Further adjustment for BMI attenuated the association (-0.067±0.028 mg•kg^{-1}•min^{-1}, p=0.026). This relationship was not confounded by renal function, blood pressure or lipid levels.

Conclusion: Serum sclerostin is inversely related to insulin-induced whole-body glucose uptake in obese but not lean women. Further studies after direct effects of sclerostin on insulin action are warranted.

Disclosure: The authors declared no competing interests.

OP5 (P392)

The Effect of Treatment with Intact PTH on Undercarboxylated Osteocalcin and Measures of Energy Metabolism in Hypoparathyroidism: a Randomised, Placebo-Controlled Trial

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Osteocalcin (OC) is produced by osteoblasts in an undercarboxylated form (ucOC). Recently ucOC has been shown to influence energy metabolism in mice. In hypoparathyroidism (HPT) secretion of parathyroid hormone (PTH) is decreased or absent suppressing bone turnover. We recently randomised patients with HPT to treatment with PTH or placebo and demonstrated a marked increase in bone turnover. In particular, OC increased by more than 800%. We therefore investigated in the same cohort if there was a similar increase in ucOC and if that increase affected energy metabolism. 62 patients with HPT were randomised to treatment with either 100 µg PTH1-84 (Preotact®, Nycomed, Denmark) or placebo for 24 weeks. We measured fat mass using DXA at baseline and after 24 weeks. We took fasting blood samples at baseline and after 24 weeks. Fasting plasma glucose was measured using standard

techniques and. By using ELISA we determined ucOC, leptin, adiponectin, and insulin. As a measure of insulin resistance we calculated HOMA-IR. During treatment ucOC increased by 1185.0±814.4% in the PTH treated group and by 69.3±79.4% in the placebo group ($p<10^{-50}$). In addition, body weight decreased by 1.1±4.0% in the treatment group and increased 0.8±2.5% in the placebo group (p=0.04). Glucose, adiponectin, leptin, HOMA-IR, total body fat mass, or truncal fat did not change significantly. Moreover, there was a significant and negative correlation between change in ucOC and change in body weight (p=0.004) or change in total body fat mass (p=0.03). Change in ucOC did not significantly correlate with changes in other parameters. In conclusion PTH treatment significantly increased ucOC and decreased body weight, however, insulin resistance or adipokines were unaffected. An explanation for the weight loos may be subtle hypercalcaemia in PTH treatment inhibiting appetite. Our data do not support a role for ucOC in energy metabolism in humans.
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OP6 (P274)

Routine Laboratory Examination in Osteoporosis in Primary Care: Uncertainty About the Benefits

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Background: Primary osteoporosis is the main cause of reduced quality of bone. In some cases however, osteoporosis is caused by an underlying disease. For this reason routine laboratory examination is advised in most guidelines. The prevalence of laboratory abnormalities in clinical setting has been established in previous studies. There is a lack of similar data in primary care populations, nor are there studies that have evaluated the medical benefit of routine laboratory examination. The goal was assessment of the prevalence and practical consequences of abnormalities in routine laboratory examination in osteoporosis in primary care.
Method: In a population study of women≥65 years with clinical risk factors for osteoporosis (n=2320), and in a population (n=2699) of persons referred for bone densitometry by GPs, we collected blood samples (including: ESR, TSH/T4, Calcium, Albumin, Creatinine and 25-hydroxyvitamin D) regardless if there was osteoporosis or not. Of all participants with one or more abnormalities, we collected data from the GPs about already existing diagnoses and previous laboratory abnormalities and the consequences for treatment in one year follow up.
Results: The prevalence of laboratory abnormalities in participants with osteoporosis (n=1334) except for 25-hydroxyvitamin D was 4.9%, which was a new finding in 1.8%. In 0.7% this influenced treatment or led to new diagnoses connected with osteoporosis. The prevalence of 25-hydroxyvitamin D≤50nmol/L was 50%. There was no association between the presence of osteoporosis or (recent) fractures and laboratory abnormalities (OR 1.00, 95%CI: 0.92-1.08, respectively OR 1.28, 95%CI: 0.88-1.87).
Conclusion: In these primary care populations the prevalence of relevant laboratory abnormalities was limited and there was no association with osteoporosis. Since all patients with osteoporosis are treated with vitamin D supplementation, the need for measurement of 25-hydroxyvitamin D is debatable. The results of this study should be a reason to reconsider the advice for routine laboratory examination in osteoporosis guidelines.
Disclosure: The authors declared no competing interests.

OP7 (P49)

Low Body Mass Index in Young Women Affects BMD and Bone Bending Strength

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Background: Adolescence and young adulthood is a critical period for the development of peak bone mass. In addition, low bone mineral density (BMD) is a frequently overlooked consequence of eating disorders in this population. Thus, bone loss that leads to fracture in the young women represents an area of active research and clinical investigation. The purpose of this study is t**o** investigate whether low BMI is associated with low bone mineral density (BMD) and bone bending strength in young women, and whether any such association could be explained by low levels of habitual physical activity, bone turnover markers or low percent body fat. The secondary purpose of this study is to examine the difference in mean BMD and bone bending strength between underweight body mass index (BMI) ≤ 18.9 kg/m^2 and normal and overweight BMI 19.0 to 29.9 kg/m^2 in young females. We hypothesised that young women with low BMI is associated with low BMD and low tibial bending strength, such association may be explained by low levels of habitual physical activity, low bone turnover marker activity or low percent body fat.
Method: Thirty females, age 18-30 years, with a BMI ≤18.9 kg/m^2 (n=15) and a BMI between 19–29.9 kg/m^2 (n=15) served as study subjects. The dependent variables are BMD and bone bending strength. BMD values were expressed as Z-score units and in absolute values for femoral neck, lumbar spine, forearm, and leg obtained with a dual-energy X-ray absorptiometry (DXA, Hologic Discovery-W scanner, Bedford, MA, USA). Bone bending stiffness of the tibia and ulna were determined using a Mechanical Response Tissue analyzer called MRTA (NASA, Mountain View, CA, USA) and bone turnover markers by ELISA using bone biomarker kits (Quidel Corporation, San Diego, CA, USA).
Results: Weight, height, FM, LBM, Body Fat % and tibia length were significantly lower (all p<0.05) in the low BMI group compared with the normal+high BMI group. Relative to the normal+high BMI group, the low BMI group also exhibited significantly lower tibial bending strength (TEI) (148 vs. 195 Nm^2, p<0.05), Femoral neck (FN) BMD (z-score -2.44 vs. 0.15), lumbar spine (LS) 1-4 BMD (z-score -2.08 vs. 0.09), whole body (WB) BMD (z-score = -0.50 vs. 0.53), forearm BMD (z-score -0.5 vs. 6.2), total hip (z-score -0.89 vs. 0.2) (all p<0.05). Multiple regression results show that significant independent predictors of TEI are total hip BMD (β = 2.51) and LS1-4 BMD (β = -1.40) (R^2 = 0.367, p<0.05). Simple correlation coefficient between ulnar bending strength (UEI) and ulna BMD (r=0.25, p=0.27) and between TEI and hip BMD (r=0.29, p=0.12) were low.

Conclusion: We concluded that young women with BMI <18.9 kg/m^2 have significantly low tibial bending strength, and low BMD for whole body, distal arm, hip, FN, and LS1-4 with z-scores ranging from -0.5 to -2.44. Total hip BMD and LS1-4 BMD were significant determinants of tibial bending strength in young females. (Funded by RSCA Mini Grant Award, Cal Poly Pomona, CA).
Disclosure: The authors declared no competing interests. This research was funded by a RSCA Mini-Grant Award of California State Polytechnic University, Pomona, California.

OP8 (P275)

The Fracture Patient Phenotype: Bone and Fall-Related Risk Factors in Patients at the Fracture Liaison Service

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Background: Fractures are the result of bone- and fall-related risk factors. We evaluated the prevalence of both bone- and fall-related risk factors in patients visiting the Fracture Liaison Service (FLS).
Methods: A retrospective chart review was performed of all consecutive patients with a recent fracture visiting the FLS for fracture risk evaluation.
Results: Out of 3,057 patients aged 50-90 years, 1,111 consecutive patients who were able and willing to be evaluated at the FLS, were included (71% women, mean age 65.2 yrs.), 8% with a hip, 29% with a major, 57% with a minor and 6% with a finger or toe fracture. At least one bone- or fall-related risk factor was present in respectively 90% and 83% of the total population. At least one fall-related risk factor was more frequently present in women (women vs. men: 86% vs. 78%, p=.002) and at higher age (80-89 vs. 50-59 yrs.: 100% vs. 76%, p=<.001). At least one bone-related risk factor was more frequently present in women (women vs. men: 91% vs. 87%, p=.043), with lower BMD (osteoporosis vs. normal BMD: 94% vs. 83%, p=<.001), with more severe fx (hip vs. finger or toe fx: 90% vs. 88%, p=.016) and with higher age (80-89 vs. 50-59 yrs.: 100% vs. 83.7%, p=<.001). Most patients had a combination of bone- and fall-related risks (77%), 12% had only bone-related risks, 6% had fall-related risks and only 4% had no bone- or fall-related risk.
Conclusion: Four out of five patients with a recent fracture presenting at the FLS have a combination of bone- and fall-related risk factors. Therefore, careful evaluation of both bone and fall-related risk factors at the FLS will contribute to optimal fracture risk evaluation and to decisions about further fall and fracture prevention.
Disclosure: The authors declared no competing interests.

OP9 (P276)

Roux-en-Y Gastrectomy Results in Greater Increase in Bone Turnover after Surgery than Sleeve Gastrectomy in Morbidly Obese Patients

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Bariatric surgery for severe obesity results in a rapid weight loss and beneficial metabolic effects, but may have negative effects on the skeleton. We evaluated the changes in bone turnover in response to bariatric surgery with two surgical techniques, Roux-en-Y gastric bypass (RYGB) and sleeve gastrectomy (SG). Forty-six morbidly obese subjects (mean age 44.9 yrs, BMI 42.1) were operated with RYGB (n=21) or SG (n=25). Twenty-five healthy subjects with normal body weight (mean age 45.8 yrs, BMI 23.0) were recruited as controls. Fasting serum samples were collected before and 6 months after the operation and bone turnover markers (CTX, PINP, TRAcP5b, osteocalcin) were analysed. Volumetric bone mineral density (vBMD) was measured by quantitative computed tomography in a subset of 21 subjects. Both RYGB and SG resulted in a significant weight loss and decrease in fasting plasma glucose and insulin (p<0.001 for all). At baseline, obese subjects had significantly lower levels of bone markers than the healthy subjects (p<0.05). Levels of bone markers increased significantly 6 months after surgery (p<0.001 for all). The median increases in CTX, PINP, TRAcP5b and osteocalcin were greater (p<0.01) after RYGB (303, 162, 72 and 141%, respectively) than after SG (106, 59, 27 and 60%). In the subset with vBMD analysis, vertebral vBMD increased in obese subjects without DM2 (N=13) (p=0.055), while there was no change in obese subjects with DM2 (N=8). Bone markers increased in all obese subjects irrespective of their DM2 status (p<0.05 for all). The surgical method did not affect the change in vBMD (p=0.60). Bone turnover is increased in response to bariatric surgery but is affected by the surgical technique. In contrary to previously published data on areal BMD by DXA, vBMD does not decrease, but may even increase at short term, in non-diabetic patients after bariatric surgery.
Disclosure: The authors declared no competing interests. This study was supported by grants from the Academy of Finland, Sigrid Juselius Foundation and Turku University Hospital Research Funds.

OP10 (P304)

The Association between Serum Uric Acid, Bone Mineral Density, Hip Bone Geometry and Fracture Risk: the role of Age and Vitamin C

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Background: We prospectively investigated the association between uric acid (UA), bone mineral density (BMD) at femoral neck (FN-BMD), hip bone geometry (HBG) parameters and incident fracture risk in elderly men and women and if these associations were modified by age and vitamin C intake.
Methods: Data of 5,074 participants of the Rotterdam Study (RS), a prospective population based cohort, were available (mean follow-up 9,9 years). Serum UA was assed at baseline. FN-BMD was measured at baseline and the 2nd, 3rd and 4th visit of the RS, whereas HBG parameters were measured at baseline and the 2nd and 3rd visit. We fitted linear regression models in generalized estimated equations to study UA in relation to FN-BMD and HBG. Cox proportional hazard regression models were used to look at the association of serum UA with fracture risk. All associations were corrected for age, gender, and confounders.
Results: Serum UA levels (per SD increase) were associated with higher FN-BMD (β=0.007, *P*=0.0002), thicker cortices (β=0.002, *P*=0.014) and lower bone width (β=-0.013, *P*=0.008). Also, UA was related to lower cortical buckling ratio (β=-0.19, *P*=0.005). Hazard Ratios (HRs) per SD increase of baseline UA levels for the development of any type of incident fractures, non-vertebral fractures and osteoporotic fractures were 0.93 (95%CI=0.86-0.995), 0.92 (95%CI=0.86-0.998) and 0.91 (95%CI=0.84-0.977), respectively. All associations were more prominent in older individuals and in participants with high intakes of vitamin C (>median) (interactions with age or vitamin C both *P*<0.05).
Conclusion: Higher levels of serum UA are associated with higher BMD (at expense of thicker cortices and narrower bone diameters) and could be a protective factor in bone metabolism among men and women. However, interactions with age and vitamin C may be present.
Disclosure: The authors declared no competing interests.

OP11 (P305)

SSRIs and Change in Bone Mineral Density in a Population-Based Study of Middle-Aged and Older Men and Women

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Selective serotonin inhibitors (SSRIs) are assumed to play a role in bone metabolism via the modulation of serotonin levels. Several cross-sectional studies reported an association between SSRI use and lower bone mineral density (BMD). However, longitudinal studies showed conflicting results and had limited longitudinal exposure information. Therefore, our objective was to investigate the association between SSRIs and BMD, and changes in BMD in a longitudinal study with 14 years of follow-up. Our study was embedded in the population-based Rotterdam Study cohort. SSRI use was based on pharmacy dispensing records. Femoral neck BMD was measured using dual-energy X-ray assessment (DXA) at minimal 1 and up to 4 visits between 1991-2004. The annual percentage change in BMD was calculated between 2 consecutive visits. Multivariate linear mixed models were applied to examine the association between SSRI use and repeated measurements of BMD, and changes in BMD, stratified by sex, in comparison to non-users. Analyses were adjusted for time-varying covariates; age, body mass index, lower limb disability, smoking, alcohol intake, medication use and depressive symptoms. The study population included 2,568 men and 3,484 women, with in total 14,488 BMD measurements and 7,212 BMD change values. At baseline, the mean age was 68.1 years (standard deviation [SD] 7.7), and 68.9 years (SD 8.3), respectively, with a median of 3.9 years between BMD measurements (interquartile range 1.8–4.6). In women, the mean BMD of SSRI users was 0.840 g/cm^2 (n=78), versus 0.831 g/cm^2 for non-users (n=6739, p=0.194). The annual decline in BMD in women was not significantly stronger in SSRI users (n=117) than in non-users (N=3086, beta= -0.093%, p=0.152). Similarly, no significant association was observed for men. Therefore, our study indicated that use of SSRIs is not associated with lower BMD, and a stronger decline in BMD in middle-aged and older men and women.
Disclosure: The authors declared no competing interests. This study was funded by the ZonMW (The Dutch Medical Research Agency) Priority Medicines Elderly program [113101002 and 1131101006; non-commercial].

OP12 (P339)

Safety and Efficacy of Odanacatib in the Treatment of Men with Osteoporosis: a Randomised Placebo-Controlled Trial

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Osteoporosis in men is an important clinical problem, associated with significant morbidity, mortality and societal expense. Odanacatib (ODN), a selective oral inhibitor of cathepsin K, is currently being investigated as a treatment for osteoporosis. This Phase III, double-blind, randomised, placebo-controlled, 24-month study investigated the safety and efficacy of ODN for the treatment of men with osteoporosis. Eligible patients were men 40–95 years of age with idiopathic osteoporosis or osteoporosis due to hypogonadism, who had a lumbar spine (LS), femoral neck (FN), or total hip (TH) T-score of ≤–2.5 to ≥–4.0 without prior vertebral fracture, or ≤–1.5 to ≥–4.0 with one prior vertebral fracture. Participants were randomised (1:1) to ODN 50 mg weekly or placebo. All received vitamin D (5600 IU/week) and calcium up to 1200 mg/day, if required. The primary efficacy outcome measure was the percent change from baseline in LS BMD. Secondary outcomes included changes in BMD at the FN, TH, and trochanter, bone turnover markers, and safety and tolerability. In total, 292 men were randomised and treated (mean age 68.8 years; 5.8% total testosterone levels <250 ng/dL). Compared with placebo, treatment with ODN for 24 months increased BMD at the LS and all 3 hip sites (TH, FN and trochanter) by 5.6%, 2.0%, 1.7%, and 2.1%, respectively (LS, TH, and trochanter $p<0.001$; FN $p=0.008$) and decreased the bone resorption marker u-NTx/Cr (–68%, $p<0.001$). Bone formation markers initially decreased with ODN, then returned towards levels found with placebo by Month 24. ODN was associated with an incidence of adverse events similar to placebo. In this study in men with osteoporosis, ODN increased spine and hip BMD, and decreased bone resorption with a smaller effect on bone formation. ODN is a promising potential therapy for the treatment of osteoporosis in men. (ClinicalTrials.gov number NCT01120600.)

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OP13 (P340)

Abstract not available

OP14 (P341)

Intranasal Administration of PTH(1-34) for the Treatment of Osteoporosis - Equivalence to Subcutaneous Injection at the Neck of Femur in the Food and Drug Administration (FDA) Mandated Preclinical Model

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Background: Osteoporosis affects 200 million people worldwide, it is characterised by low bone mass and micro-architectural deterioration, increasing risk of fracture. PTH(1-34) is a proven anti-osteoporotic drug, self-administered by subcutaneous (SC) daily injection. Patient compliance to PTH(1-34) therapy can be sub-optimal for some patients. There is therefore an urgent unmet clinical need to offer alternative administration regimens. Intranasal (IN) delivery of PTH(1-34) offers an attractive non-invasive approach to improve compliance. We show, for the first time, using a proven intranasal nano-enabled delivery system, an equivalent anabolic effect on bone with the same dose of PTH(1-34) administered either SC or IN. The aim was to determine the anabolic effect of PTH(1-34) delivered intranasally in the US FDA pre-clinical model for research into osteoporosis.

Methods: PTH(1-34) liquid formulations for IN delivery were analysed for stability. Following ethical approval, ovariectomised (OVX) rats were randomly divided into groups receiving an equal dose of SC or IN PTH(1-34). Bone tissue was assessed using micro computed tomography following ovariectomy or SHAM and treatment with PTH(1-34) IN or SC, or no PTH(1-34) IN and SC controls. Data were subject to Kolomogorov-Smirnov tests for normality followed by ANOVA.

Results: Stability of formulations was confirmed using HPLC analysis >12 months. Ovariectomy induced bone loss was confirmed in neck of femur (NOF) trabeculae: 50% reduction in bone volume, 51% reduction in trabecular number and 37% increase in trabecular porosity ($p<0.05$). Post treatment NOF trabecular bone volume significantly increased 58% (OVX 21.22±1.6% vs IN 33.5±2.5%) trabecular number significantly increased 50% (OVX 3.27±0.2mm^{-1} vs IN 4.91±0.29mm^{-1}) and trabecular porosity significantly decreased 16% (OVX 78.78±1.6% vs IN 66.50±2.5%) ($p<0.05$). There was no significant difference in bone microarchitecture between SC and IN administration of PTH(1-34) at equivalent doses ($p>0.05$).

Conclusion: Intranasal delivery of PTH(1-34) is a viable alternative to subcutaneous injection to improve patient compliance.

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OP15 (P342)

Early Changes in Bone Turnover Markers are Associated with Increases in BMD During Treatment with Blosozumab in Postmenopausal Women with Low BMD

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Background: A randomised, blinded study evaluated the effect of treatment with blosozumab, a humanised monoclonal antibody targeted against sclerostin, on BMD and bone turnover markers (BTMs) in postmenopausal women with low BMD. The objectives of these analyses are to determine the 1) relationship between change in BTMs (PINP, BALP, OC, and CTX) at 2, 4, and 12 weeks and change in BMD after 1 year of treatment; 2) BTM and time point most strongly associated with 1-year change in spine, hip, and total body BMD; 3) proportion of patients responding to treatment based on change in BTMs.
Method: Multiple regression models with applied forward selection identified the strongest association between change in BTM and change in spine, hip, and total body BMD after 1 year of treatment. Least significant change (LSC) in BTM was determined from within subject variability of repeated measures to define proportion of responders.
Results: A strong association was observed between early change in BTMs reflecting bone formation and BMD increases with 1-year of blosozumab treatment. In a model considering treatment effect and BTMs reflecting bone formation, changes in PINP at 2 and 4 weeks were significantly correlated with changes in spine ($p<.01$) and hip BMD ($p<.02$) at 1 year. When treatment and PINP increases are considered, CTX decrease at 2 weeks was also significantly correlated with change in hip ($p=.04$) but not spine BMD. In the highest dose group, PINP at 4 weeks was correlated ($p<.01$) with BMD at 52 weeks (spine $r=.51$, hip $r=.56$, whole body $r=.62$). The LSC from post-baseline PINP measurements in the placebo group was 10 ng/mL; response rates were >95% with Q2W dosing, 52% with Q4W dosing, and 8% with placebo.
Conclusion: We conclude change in PINP by 4 weeks of treatment with blosozumab identifies later BMD response at 1 year of treatment.
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OP16 (P393)

Osteoblasts from Type V OI Patients Demonstrate Gain-of-Function for Mineralisation Despite Decreased *COL1A1* Expression

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Osteogenesis imperfecta (OI) is a genetically heterogeneous disorder characterised by bone fragility. Most cases result from dominant mutations in type I collagen, while recessive OI is caused by defects in genes whose products interact with type I collagen. Type V OI has dominant inheritance, with characteristic skeletal findings and mesh-like lamellation on bone histology. It is caused by a unique heterozygous mutation in *IFITM5* (c.-14C>T), which encodes BRIL, a transmembrane protein expressed in osteoblasts. The mutation generates a start codon in the 5'-UTR, adding five residues to the BRIL N-terminus. However, the mechanism of type V OI and its relationship with type I collagen is unknown. We identified 8 patients with the *IFITM5* (c.-14C>T) mutation. Using cultured osteoblasts from patients with characteristic type V OI, we verified expression and stability of mutant *IFITM5* transcripts. In differentiated type V OI primary osteoblasts in culture, *IFITM5* expression and BRIL protein level is comparable to control. Both early (*ALPL* and *IBSP*) and late (osteopontin and osteocalcin) markers of osteoblast differentiation are increased in type V OI osteoblasts. Mineralisation, assayed by alizarin red staining, was increased in type V OI osteoblasts compared with control. In contrast to other differentiation markers, type V OI osteoblasts have less than half the level of *COL1A1* transcripts found in control in mid to late differentiation, with concomitantly decreased collagen protein secretion. Decreased secreted type I collagen underlies decreased crosslinked collagen in matrix, and altered appearance of fibrils deposited in culture. The increased mineralisation and advanced differentiation of type V OI osteoblasts likely underlie the overactive tissue calcification and hypertrophic callus formation seen in affected individuals and demonstrates that type V OI has a gain-of-function mechanism. Decreased type I collagen expression, secretion and matrix incorporation establish type V OI as a collagen-related defect.
Disclosure: The authors declared no competing interests.

OP17 (P394)

Non-Surgical Hypoparathyroidism in Denmark – Epidemiology, Mortality and Complications

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Background: Non-surgical hypoparathyroidism (NS-HypoPT) is a rare disease, characterised by low levels of calcium and PTH. A number of genetic variants have been shown to cause inadequate PTH secretion, although the aetiology often remains unknown. It may also appear on autoimmune basic, either isolated, as a part of the autoimmune polyendocrine syndrome, associated with APS-1, or as acquired antibodies that activate the calcium sensing receptor (CaSR). Autosomal dominant hypocalcaemia (ADH) is caused by an activating mutation (gain-of-function) in the CaSR. Little is known about this group of patients, including their mortality and morbidity. The aim was to identify all patients diagnosed with NS-HypoPT in Denmark and assess their mortality and risk of complications.
Methods: Cases (patients with NS-HypoPT) were identified through registers and review of their individual hospital charts. To access their mortality and morbidity we compared the cases with a group of age-and gender matched population based controls.
Results: In a population of 5.336.394 persons, a total of 180 cases with NS-HypoPT were identified, among whom 123 (68%) were alive at the day of follow-up, equal to a prevalence of 2.3/100,000 inhabitants). Only 38 were genetic verified. Compared with controls, mortality was not increased, but patients had a significantly increased risk of seizures (Hazard ratio [HR] 10.05) renal insufficiency (HR 6.01), cataract (HR 4.21), neuropsychiatric complications (HR 2.45), infections (HR 1.94), cardiovascular diseases (HR 1.91) and fractures at the upper extremities (HR 1.93). In contrast, patients had significantly reduced risk of malignant diseases (HR 0.44).
Conclusion: NS-HypoPT is a rare disease associated with a number of complications that should be considered when taking care of these patients.
Disclosure: The authors declared no competing interests.

OP18 (P395)

Baseline Characteristics of the ZIPP Study Cohort Provide a Unique Insight into the Evolution of *SQSTM1* Mediated Paget's Disease

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Paget's disease of bone (PDB) is a common disorder with a strong genetic component. The most important susceptibility gene is SQSTM1. Mutations of this gene are found in about 40% of patients with familial and 10% of those with sporadic PDB but there is limited information on the early characteristics of disease evolution in *SQSTM1* mutation carriers Here we report on the baseline clinical characteristics of participants of the ZIPP study - a multinational randomised trial of genetic testing and targeted intervention with Zoledronic acid in asymptomatic subjects who carry *SQSTM1* mutations. The study group comprised 203 *SQSTM1* mutation positive subjects of mean (±SD) age 49.2 ± 9.0 years of whom 110 (54.4%) were female. The most common mutation was P392L (63.5%) followed by M404V (12.3%), G425R (9.9%), A390X (3.9%), G411S (3.4%), Glu396X (1.5%), Thr350GlnfsX28 (1.5%), Gln371X (1%), F406V (1%), K378X (1%), E396X (0.5%) and I424S (0.5%). Analysis of radionuclide bone scan images at baseline revealed abnormalities that were thought to represent early PDB-like lesions in 31 subjects (15.2%). The commonest sites were the spine (48%), pelvis (35%), femur (12%) and tibia (6.4%). About 30% of subjects had multiple lesions. All subjects were asymptomatic. Serum total alkaline phosphatase (ALP) concentrations were elevated in 10 subjects (5%). Subjects with PDB-like lesions were marginally older than those without lesions 51.1 ± 9.4 *vs.* 49.2 ± 8.9 and lesions were significantly more common in men (20.6% vs. 10.9% (p=0.02). Elevated ALP concentrations were found in 16% of subjects with lesions and 3% of subjects without lesions (p=0.002). There was no association between *SQSTM1* mutation type and the presence of lesions. This study demonstrates that by the age of 50 about 15% of *SQSTM1* mutation carriers have asymptomatic PDB-like bone lesions. Lesions occur more commonly in men, which is consistent with the fact that PDB affects men more frequently than women. The study confirms that PDB is a clinically silent disease in its early stages and shows that measurements of total ALP are not a sensitive means of detecting early lesions. Further follow up of this cohort will provide a unique insight into the evolution of PDB with age and into the effects of zoledronic acid in modifying the natural history of this condition.
Disclosure: Consultancy to institution for Merck and Novartis. Research funding from Novartis, Amgen, Eli Lilly, Arthritis Research UK and Medical Research Council.

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ORAL POSTERS – PRE-CLINICAL

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OP21 (P224)

UBR5, an E3 Ubiquitin-Protein Ligase, Regulates Hedgehog-mediated Tendon Ossification

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The objective was to determine the role of Hedgehog signalling in UBR5-associated heterotopic tendon ossification. Our studies into the N-end Rule Ubiquitin-protein ligase UBR5 revealed its role in controlling heterotopic tendon ossification in the mouse limb and hypothesise that UBR5 regulates stem/progenitor behaviour to control tendon homeostasis. Spatiotemporal skeletal development is tightly regulated to maintain a functioning skeleton. However, in certain diseases heterotopic ossification (HO) can occur in soft tissue. Using Prx-Cre combined with a floxed UBR5 mutant allele (UBR5mt) we deleted UBR5 function in the developing murine embryonic limb bud. Micro-CT analysis revealed HO in homozygous UBR5mt adult animals and histological analysis identified numerous chondrocytes within the tendon midbody. HO was progressive, identified in multiple tendons and first detected at 6 weeks of age. Importantly, no HO was observed in the Prx-Cre control animals (n=18). Our work in other tissues indicates UBR5 as an important regulator of stem/progenitor cell function, with UBR5 being highly upregulated in pericytes, the progenitors of mesenchymal stem cells. Furthermore, we revealed that UBR5 regulates both Hedgehog (HH) ligand production and signal transduction. Indian Hedgehog signalling plays a central role in controlling stem/progenitor cell function in various tissues including the adult skeleton. UBR5mt animals treated with the HH pathway antagonist cyclopamine (n=3) resulted in an enhanced level of HO in comparison with those treated with an inactive cyclopamine analog (n=4). Cyclopamine treatment in a wild-type background did not promote HO, suggesting that a UBR5mt background sensitises tendons to HH pathway inhibition and ossification. We therefore conclude that UBR5 and HH signalling normally act to suppress heterotopic tendon ossification. In conclusion, UBR5 regulates heterotopic tendon ossification through regulating HH signalling.
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OP22 (P79)

Oxidative Stress Inhibits PTH Type 1 Receptor Signalling and Trafficking

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During ageing, an increase of reactive oxygen species (ROS) occurs, affecting several processes involved in bone homeostasis, namely osteoblast and osteoclast apoptosis, osteoblastogenesis and adipogenesis. Various signalling pathways are known to be enhanced or decreased by ROS and constitute potential therapeutic targets to limit oxidative damage effects in ageing-associated diseases. Transient administration of parathyroid hormone (PTH), a master regulator of bone remodelling, currently represents the only anabolic therapy in osteoporosis. However, the molecular mechanisms underlying the anabolic features of PTH are ill defined. In osteoblasts, PTH binds to the PTH type 1 receptor (PTH1R), a G protein-coupled receptor, and triggers classic G-protein signalling pathways. The aim of the present study was to analyse the effects of ROS on PTH1R signalling and trafficking. We used fluorescence resonance energy transfer (FRET)-based cAMP (Epac) and ERK biosensors, and the calcium fluorescent dye Fluo-4 to analyse by microscope live cell imaging cAMP, ERK and calcium signalling, respectively, triggered by PTH (1-34) in PTH1R-overexpressing human embryonic kidney (HEK)-293 cells in the presence or absence of H_2O_2. PTHR internalisation and recycling was measured in HEK-293 cells transiently transfected with HA-PTHR using an ELISA protocol based on an anti-HA antibody and an anti-IgG conjugated with alkaline phosphatase. An increase in cAMP production, ERK phosphorylation and accumulation of intracellular calcium was observed upon PTH (1-34) stimulation of HEK-293 cells. Preincubation of these cells with 1-500 μM H_2O_2 substantially inhibited all of these PTH (1-34)-dependent signalling pathways. These inhibitory effects were not a result of PTH (1-34) oxidation since PTH (1-34) incubated or not with H_2O_2 triggered similar cAMP responses. In addition, PTH (1-34) ligand induced about 25% internalisation and subsequent recycling of the PTH1R, and both events were significantly reduced by H_2O_2 preincubation in these cells. These findings highlight the role of H_2O_2 as an inhibitor of PTH signalling, and suggest the relevance of ROS as a putative target in bone diseases associated to oxidative stress such as age-related osteopenia.
Disclosure: The authors declared no competing interests.

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OP23 (P14)

A Stable Synthetic Sulforaphane Significantly Improves Bone Architecture and Gait in the Naturally Occurring Str/ort Model of Osteoarthritis

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Osteoarthritis (OA), affecting joints and bone, causes physical gait disability with huge socio-economic burden; treatment remains palliative. Roles for antioxidants in preventing/reversing such chronic disorders have been examined previously. Sulforaphane is a naturally occurring antioxidant inflammation modulator. Herein, we explore whether Sulforadex®, a stable synthetic form of sulforaphane, modifies gait, bone architecture and slows/reverses articular cartilage destruction in a spontaneous OA model in Str/ort mice. Sixteen mice (n=8/group) were orally treated for 3 months with either 100 mg/kg Sulforadex® or vehicle (0.5% sodium carboxymethyl cellulose in H_2O). Gait was recorded and analysed using a DigiGait imaging system. Tibiae were microCT scanned using a Skyscan 1172 and architecture in a defined trabecular bone region and the entire cortical shaft analysed. Right knees were decalcified, wax-embedded and multiple 6µm coronal sections collected from across each entire joint stained with toluidine blue. OA lesion severity was graded using an Internationally recognised system. Analysis revealed development of asymmetric gait (hind limb paw area), normally linked to OA, in vehicle-treated Str/ort mice, which did not emerge in Sulforadex®-treated mice. Fore-limb asymmetry followed similar patterns. We found significantly increased trabecular bone volume/total volume, bone surface and trabecular number in Sulforadex®-treated mice. This was consistent with significantly increased indices of bone strength (cross-sectional thickness, Imin/Imax in many regions along the tibial shaft. Despite these marked improvements in gait and superior trabecular and cortical bone mass and architecture, we found that histologically-graded OA severity in articular cartilage was unmodified in the Sulforadex®-treated mice. This indicates that Sulforadex® improves bone microarchitecture and indices of mechanical strength and produces greater symmetry in gait without any marked effects on cartilage lesion development in these spontaneously osteoarthritic mice. Our findings support novel osteotrophic roles for Sulforadex® and beneficial gait effects that appear to be independent of articular cartilage lesion development in OA.

Disclosure: The authors declared no competing interests.

OP24 (P140)

Skin Inflammation Causes Bone Loss by IL17A-Mediated Inhibition of Bone Formation

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Patients with chronic inflammatory diseases such as psoriasis are at high risk for developing osteoporosis. Psoriatic arthritis patients exhibit bone loss caused by increased bone resorption through activation of osteoclasts. However, it is not clear whether psoriasis can lead to bone loss in the absence of arthritis. Using mouse models with skin inflammation as well as psoriasis patient samples, we show that increased circulating IL-17A from the inflamed skin triggers bone loss through inhibition of bone formation. Osteocalcin (OCN), P1NP levels as well as bone formation rates were decreased in mice with an epithelial (Keratin5)-specific deletion of JunB (JunB$^{\Delta ep}$). Moreover, transgenic mice expressing IL-17A in keratinocytes exhibit decreased OCN and P1NP levels with no changes in TRAcP5 levels. Expression of Phex, Dmp1 and Sost, markers of osteocytes, were altered in both models of skin inflammation. The inhibition of bone formation by IL-17A was independent of its expression in T-cells, since JunB$^{\Delta ep}$ mice on a Rag-/- background displayed decreased levels of OCN. Importantly, pharmacologic IL-17A blockade rescued Ocn expression and bone formation rates in JunB$^{\Delta ep}$ mice. Mechanistically, IL-17A inhibits osteoblast maturation and mineralization *in vitro*. RNA-seq analyses from *in vitro* osteoblast cultures treated with IL-17A identified nitric oxide and lipocalin-2 (Lcn-2) as mediators of IL-17A-dependent osteoblast inhibition. *In vivo*, crossing JunB$^{\Delta ep}$ mice to Lcn-2-deficient mice reduced bone loss. Importantly, psoriasis patients without arthritis developed bone loss with decreased OCN levels and increased serum IL-17A levels. Therefore, this study suggests that IL-17A, upregulated in inflammatory and autoimmune diseases, provides a risk for bone loss and its blockade should be considered in such diseases to prevent the adverse consequences on the skeleton.

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OP25 (P141)

DLX3 is a Major Regulator of Bone Apposition and Homeostasis in the Appendicular Skeleton

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Although human mutations and *in vitro* studies suggest that DLX3 is involved in bone formation, its *in vivo* role has not been elucidated. To address the functions of DLX3 in the appendicular skeleton, we generated and analysed mice carrying conditional loss-of-function mutation of DLX3 in osteoblasts (*Dlx3*$^{OCN\text{-}cKO}$). Using dynamic bone formation, histological and micro-computed tomography analyses, we demonstrated that *in vivo* DLX3 deletion in osteoblasts results in significant increase in bone mass throughout the lifespan. In absence of DLX3, endochondral bone formation still takes place at the growth plate but we observed more trabeculae that extend deeper into the medullary cavity. Furthermore *Dlx3*$^{OCN\text{-}cKO}$ cortical bone is thicker with higher mineral apposition rate, decreased bone mineral density and increased cortical porosity. By combining *in vivo* site-specific gene profiling, TRAP staining and *ex vivo* culture of M-CSF-dependent mononuclear cells, we showed that the increase in trabecular bone mass in *Dlx3*$^{OCN\text{-}cKO}$ mice does not arise from impaired osteoclastic activity but from direct enhancement of bone-forming osteoblast activity with an imbalance in bone homeostasis in favour of bone apposition. *In vivo* RNA-seq analysis on *Dlx3*$^{OCN\text{-}cKO}$ metaphysis demonstrated that DLX3 deletion in osteoblasts results in up-regulation of genes encoding transcription factors essential for osteoblastogenesis as well as genes important to mineral deposition and bone turnover. Finally, using DLX3-deleted bone marrow stromal cells and ChIP-seq analysis, we demonstrated that DLX3 removal results in increased osteoblast differentiation associated with enhanced occupancy of key transcriptional activators of osteogenesis on the bone-specific osteocalcin (OCN) promoter. In conclusion, these results demonstrate for the first time that DLX3 plays a central role in the maintenance of bone homeostasis and skeletal integrity by attenuating bone mass accrual in the appendicular skeleton.

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OP26 (P142)

VEGF-Dependent Control of Osteoblast/Adipocyte Differentiation

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Vascular endothelial growth factor A (VEGF) functions as a key factor in angiogenesis but also plays essential roles in cellular survival, cartilage and bone development, and bone maintenance. Apart from coupling angiogenesis and osteogenesis, VEGF regulates osteoblast progenitor cell fate by controlling the balance between osteoblast and adipocyte differentiation. Conditional deletion of VEGF in Osterix-expressing osteoblast progenitor cells in mice, carrying floxed *Vegfa* alleles and the Osx-Cre transgene, leads to age-related osteopenia characterised by loss of bone mass with increased marrow fat. Both *in vivo* and *in vitro* studies indicated that VEGF knockdown induces differentiation of mouse bone marrow stem cells (BMSCs) into adipocytes at the expense of osteoblasts. Experiments aimed at rescuing differentiation defects revealed that addition of exogenous recombinant VEGF had no effect on BMSC fate, suggesting that VEGF functions via intracrine rather than paracrine mechanisms. A role for intracellular VEGF was supported by detection of VEGF and VEGF receptors in nucleus and cytoplasm using immunostaining and western blotting of BMSC subcellular fractions. To assess the effect of modulating intracellular VEGF levels on BMSC differentiation, we generated a cell-permeable VEGF protein consisting of VEGF fused to TAT, a cell-penetrable peptide, followed by a nuclear localisation sequence. This cell-permeable VEGF proved to be effective in entering cells and translocating to their nucleus. Remarkably, cell-permeable VEGF, but not paracrine VEGF, stimulated expression of osteoblast marker genes in BMSCs. VEGF protein levels in nucleus and cytoplasm appeared to be regulated by mechanisms involving proteasome activity. In summary, we have identified a novel function for VEGF in controlling the fate of BMSCs involving intracrine mechanisms that are independent of its role as secreted growth factor. (Animal experiments were approved by Harvard Medical Area Standing Committee and in agreement with U.S. Public Health Service Policy on Humane Care and Use of Laboratory Animals.)

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OP27 (P186)

Gene Correction by Homologous Recombination in *TCIRG1*-Defective Induced Pluripotent Stem Cells

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Autosomal Recessive Osteopetrosis caused by mutations in the *TCIRG1* gene, is a severe bone disorder characterised by

bone marrow fibrosis and consequent pancytopenia, multiple spontaneous fractures, blindness and hearing loss. The *oc/oc* mouse well recapitulates the clinical signs of the disease. To date, haematopoietic stem cell (HSC) transplantation is the unique possible treatment, however the chance of cure is limited by the need for a matched donor. With the final aim to exploit novel therapeutic strategies allowing the use of corrected autologous HSC, we evaluated the feasibility and potentiality of induced pluripotent stem cells (iPSc), as alternative and unlimited source of autologous stem cells. To this end, we reprogrammed murine wild-type (wt) and *oc/oc* fibroblasts into iPSc, to genetically correct the *Tcirg1* mutation by homologous recombination, and to generate haematopoietic stem and progenitor cells able to give rise to functional osteoclasts. We employed a third generation polycistronic lentiviral vector carrying the reprogramming genes Oct4, Sox2 and Klf4, subsequently excisable by the Cre recombinase. After reprogramming, iPS clones with low vector copy number and normal numerical distribution of chromosomes were treated with Cre and sub-cloned. Obtained iPSc showed normal karyotype and pluripotency tested by teratoma formation assay, *in vitro* embryonic germ layers differentiation, and expression of stemness markers by immunocytochemistry and RT-PCR. Importantly, iPSc were successfully derived from *oc/oc* fibroblasts, and then corrected through homologous recombination upon transfection with a BAC containing the wt gene. iPSc were guided to differentiate towards haematopoietic belonging to different lineages. We obtained differentiation towards osteoclasts, the relevant cells in our model, which were functional as demonstrated by the dentine resorption assay. In conclusion, we provided the first evidence of targeted gene correction in osteopetrotic iPSc, supporting the rationale of using iPSc as future source of donor cells in the clinical setting.

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OP28 (P187)

Abstract withdrawn

OP29 (P15)

In Vivo Bone Surface Monitoring Reveals Age-Related Changes in Adaptive Bone (Re)Modelling Sequences

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Age-related bone loss is associated with a failure in bone (re)modelling (modelling and remodelling) processes. Bone (re)modelling occurs at spatially and temporally discrete sites to remove damaged or older bone, replacing it with new bone. However, the evaluation of bone (re)modelling has mainly been retrospective. The aim of this study was to identify the time kinetics of (re)modelling in response to loading at different ages. The left tibiae of female C57Bl/6J mice (10 wks: n=6, 26 wks: n=13, 78 wks: n=10) underwent two weeks of in vivo cyclic compressive loading [1]. The right tibia served as control. In vivo microCT at an isotropic voxel resolution of 10.5 µm was performed at the tibial mid-shaft (5% tibia's length; day 0, 5, 10, 15). Images were registered, binarised, and segmented. Two consecutive images in a common coordinate system were compared (day k–day k+1) to identify formation (F), resorption (R) and quiescence (Q) sites on the cortical endocortical and periosteal bone surfaces [2]. Comparing the three time intervals (d0–d5, d5–d10, d10–d15), 27 (re)modelling sequences (FFR, RRF, QQF, etc) were identified. Using this dynamic micro-tomography based technique we could show that during two weeks of skeletal loading, bone adaptation occurs predominantly by modelling-bases formation and resorption processes, which last less than 10 days. In young and adult bones adaptive formation processes can be extended to a longer time-interval, whereas the elderly mice lost this ability. Ageing reduced adaptive modelling (spatially unlinked formation and resoprtion) and increased remodelling (resorption followed by formation). This *in vivo* approach of tracking local movements of the endosteal and periosteal bone surface allows not only to detect how strong the local response to mechanical loading is, but when it sets in and how long it lasts. This should be of great help to find an adequate stimulation – not only mechanical, but also pharmaceutical – that results in a sustained response of bone formation on the cortical surfaces.

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OP30 (P216)

Evidence that the Human *SOST* Gene is 1α,25-Dihydroxyvitamin D Sensitive

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Sclerostin, the *SOST* gene product, is a negative regulator of bone formation and a positive regulator of bone resorption. In a screen to identify novel regulators of *SOST* expression, we found that treatment of human primary osteoblasts with 1α,25-dihydroxyvitaminD_3 (1,25D) resulted in increased expression of *SOST* mRNA and sclerostin protein. This effect was also evident in the human osteosarcoma/osteocyte-like cell line SAOS2. Effects on *SOST* mRNA levels occurred as early as 3 hours post-stimulation, consistent with a direct effect of 1,25D on the *SOST* promoter. Sequence analysis of the published human *SOST* gene revealed a single putative vitamin D response element (VDRE) upstream of the transcription start site (TSS). Cloning of this sequence into a luciferase reporter construct upstream of the constitutive thymidine kinase (TK) promoter, and transfection into HEK-293T cells, identified the presence of a 1,25D responsive element with activity equivalent to that of the well characterised mouse osteopontin VDRE. Electrophoretic mobility shift analysis (EMSA) of HEK-293T nuclear extracts revealed a 1,25D dependent gel-shift, consistent with binding of the VDR/RXR heterodimeric com-

plex. Sequence substitution in the VDR/RXR half-sites abolished VDRE reporter activity and binding of nuclear proteins. In addition, transient expression of a 6.3 kb fragment of the proximal *SOST* promoter ahead of the TSS in a luciferase expression vector demonstrated a promoter responsive to 1,25D. However, addition of a known bone specific enhancer region ECR5 ahead of the cloned promoter fragment did not increase the level of responsiveness to 1,25D. Mutation or deletion of the predicted VDRE resulted in the 6.3 kb *SOST* promoter being unresponsive to 1,25D. We conclude that 1,25D is a direct regulator of *SOST* gene expression, extending the pathways of control of sclerostin expression.

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OP31 (P217)

Estradiol Modulates and Recovers Osteocyte Metabolic/Lipid Profiles after Ovariectomy in Acute and Long-Term *In Vivo* Hormone Replacement Therapeutics

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For the first time, we assessed the metabolic and lipid profiles of osteocytes *ex vivo*. During menopause, the appearance of an osteoporotic condition can be associated with an overall metabolic decline in bone cells, and we hypothesised that it is mainly attributed to osteocyte metabolic and lipid changes, which are attenuated after increasing blood oestradiol (E2) levels. To test this, we considered control and ovariectomised (OVX) female rats in order to compare metabolic/lipid profiles of bone-embedded osteocytes, in the presence or absence of E2. Animal groups (used accordingly with FELASA approved procedures) were: a) Controls SHAM, CTL; b) ovariectomised animals, OVX; and c) OVX+E2 (single bolus injection 30 µg/Kg, 24 hours prior sacrifice for the acute study. For the subchronic study, rats were implanted with 0.5mg E2 slow release pellets for 21 days. 24 hours prior sacrifice, animals were I.P. injected with deuterated water for metabolic fluxes analyses. Left and right femurs and tibia were surgically removed and freeze-clamped or preserved for DXA and µCT analysis. Extracted metabolites from those cells as well as total lipids were analysed by ^{1}H nuclear magnetic resonance (NMR) spectroscopy and ^{2}H NMR for *de novo* lipogenesis analyses. Total lipids were extracted, quantified and analysed by HPLC-MS and fatty acids analysed by GC-MS. Ovariectomy clearly changed lipid profile inducing significant changes in both diacyl- and choline-plasmalogens content (comparatively with SHAM and OVX+E2 groups). Also, an increase of the relative proportion of long chain fatty acids was observed in the OVX group, being attenuated by 24h-treatment with E2. Total lipids analysis revealed that E2 was able to recover the CTL profile in OVX+E2, and decrease *de novo* synthesis of lipids after 21 days treatment. In terms of metabolites profile, the OVX group presented a slight decrease of lactate/alanine ratio, although osteocytes were forced to produce high levels of lactate after E2 treatment, increasing this ratio. Our results show a change in the process of lipid remodelling as a result of ovaries removal, with E2 partially compensating the alteration in long chain fatty acids. High levels of PC-Plasmalogens measured in OVX animals may be related to a signalling/protective action against the damaging effects of oxidative stress triggered by the E2 decline. Acute E2 administration in OVX animals induced osteocytes to increase aerobic glycolysis in an attempt to compensate for the metabolic deficit associated with ovaries removal. Long-term therapeutics supported the effects of the acute study, indicative of the high impact of this hormone in osteocytes metabolism.

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OP32 (P445)

Plasminogen Activator Inhibitor-1 is Involved in Glucocorticoid-Induced Osteopenia, Diabetes and Muscle Wasting in Mice

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Glucocorticoids (GC) have been widely used for the treatment of inflammatory disease. Despite high efficacy of GC treatment, its clinical use is limited by the numerous adverse effects, including osteoporosis, diabetes and muscle wasting. However, its pathogenesis remains unclear, and the evidence for systemic mediators in GC effects are lacking. Plasminogen activator inhibitor-1 (PAI-1) is adipocytokine, which is induced by GC treatment. Previous studies suggest that elevated circulating PAI-1 level is associated with several metabolic disorders, such as diabetes and osteoporosis. Therefore, in the present study, we examined the role of PAI-1 in GC-induced osteoporosis, glucose/lipid abnormalities and muscle wasting by using PAI-1-deficient mice. GC treatment for 4 weeks markedly increased the levels of circulating PAI-1 and PAI-1 mRNA in white adipose tissues in wild-type mice. Quantitative CT and histological analysis revealed that PAI-1 deficiency blunted GC-induced bone loss and the number of osteoblasts decreased by GC treatment in tibia of mice. Moreover, exogenous PAI-1 treatment induced apoptosis in primary os-

teoblasts obtained from mouse calvaria in vitro, suggesting that PAI-1 deficiency protects from GC-induced bone loss presumably through a decrease in apoptosis of osteoblasts. PAI-1 deficiency significantly improved insulin resistance but not hyperlipidaemia induced by GC treatment in mice. In vitro study revealed that exogenous PAI-1 treatment inhibits insulin-induced phosphorylation of Akt and glucose uptake in hepatocytes, but not in adipocytes and myotubes, suggesting that PAI-1 is involved in GC-induced insulin resistance by affecting hepatocytes. Moreover, PAI-1 deficiency blunted GC-induced muscle loss in mice. In conclusion, we first demonstrated that PAI-1 is involved in the metabolic adverse effects of GC treatment, such as bone loss, insulin resistance, and muscle wasting in mice. PAI-1 may be a novel therapeutic target for decreasing GC-induced adverse outcomes and also a diagnostic marker of GC-induced osteoporosis, diabetes and muscle wasting.
Disclosure: The authors declared no competing interests.

OP33 (P225)

HIF1α Down-Regulates MMP-13 Expression through Blockade of Wnt Canonical Signalling

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Background: Chondrocyte catabolism and MMP-13 expression are triggered by activation of Wnt pathway in osteoarthritis (OA) along with a loss of hypoxic environment. The mechanism by which Wnt/β-catenin pathway is lost down-regulated physiologically is unknown. We speculated that Hypoxia Inducible Factor 1α (HIF1α) regulates Wnt activation and catabolism. Here we investigated the effect of HIF1α/β-catenin interaction in the regulation of MMP-13 expression in OA.
Methods: Murine chondrocytes from WT and ΔHIF1α were cultured with Wnt3a in 21% O_2 and 1% O_2 (hypoxic) and we analysed the expression of the catabolic markers. The binding of TCF4 to *MMP13* regulatory region was assessed by Chip assay. To determine the role of this interaction *in vivo*, $\Delta HIF1\alpha^{chon}$ and $HIF1\alpha^{fl/fl}$ mice underwent DMM and received articular injection of PKF 118-310, an inhibitor of β-catenin/TCF4 interaction.
Results: Hypoxia abolished the Wnt induced decrease of *COL2* and the increase of *MMP13* expression. HIF1α knockout enhanced the expression of *Mmp13* while HIF1α over-expression inhibited it. In hypoxic chondrocytes, Chip assay reveals that HIF1α lowered β-catenin/TCF4 binding to Mmp13 regulatory region by interacting directly with β-catenin. Induced OA resulted in a decreased HIF1α expression in articular cartilage of WT mice. Furthermore, DMM in $\Delta HIF1\alpha^{chon}$ mice induce more sever cartilage lesions with higher expression of β-catenin and Mmp13. Local administration of PKF 118-310 prevented cartilage lesions and reduced Mmp13 expression.
Conclusion: Here, we show that HIF1α prevents cartilage degradation through blockade of β-catenin/TCF4 binding and decrease of Mmp-13 expression. HIF1α is an inhibitor of Wnt signalling and should be targeted in OA to reduce chondrocyte catabolism.
Disclosure: The authors declared no competing interests.

OP34 (P226)

UBR5, an E3 Ubiquitin-Protein Ligase, Regulates Hedgehog-mediated Articular Cartilage Homeostasis

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Our objective was to investigate UBR5's role in regulating articular cartilage homeostasis. Our work has revealed the N-end Rule Ubiquitin-protein ligase UBR5 as a potent suppressor of osteoarthritis-associated changes in murine articular cartilage (AC). Using Prx-Cre combined with a floxed UBR5 mutant allele we deleted UBR5 function in the developing murine limb buds. Homozygous UBR5 mutant (UBR5mt) limbs appeared morphologically normal, but exhibited reduced Hedgehog signalling (*IHH, PTCH1, GLI1*) and perturbed expression two master regulators of chondrocyte biology (*MSX2* and *RUNX2*). Six-week-old UBR5mt animals exhibited chondrocyte clustering, massively increased numbers of hypertrophic-like chondrocytes, osteophytes, vascular invasion and cartilage fibrillation (n=6). By 12 weeks of age, UBR5mt animals exhibited dramatic AC loss down to the subchondral bone (n=6). We hypothesise that UBR5 influences stem/progenitor-mediated control of AC homeostasis. Our work in other murine tissues indicates UBR5 as an important regulator of stem/progenitor cell function, with UBR5 being highly upregulated in pericytes, the progenitors of mesenchymal stem cells. Furthermore, we revealed that UBR5 regulates both Indian Hedgehog (IHH) ligand production and signal transduction. IHH-mediated signalling plays a central role in governing stem/progenitor cell function in various tissues, including juvenile and adult bone. Based on IHH's role in the growth plate, we hypothesise that UBR5 normally functions to promote IHH-mediated suppression of chondrocyte hypertrophy and AC homeostasis. Our current work addresses this hypothesis by (i) utilising Hedgehog pathway agonists and antagonist and (ii) Hedgehog signalling-associated gain-and loss-of-function alleles to modify the UBR5mt AC phenotype. We conclude that UBR5 regulates AC homeostasis and suppresses chondrocyte hypertrophy.
Disclosure: The authors declared no competing interests. This work was supported by The University of Edinburgh.

OP35 (P227)

PiT1/Slc20a1 Mediates Survival of Chondrocytes from Endoplasmic Reticulum-Induced Stress *In Vivo* and *In Vitro*

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The synthesis of an abundant extracellular matrix (ECM), together with a harmful microenvironment triggers an evolutionary conserved mechanism known as the unfolded protein response (UPR). Activation of UPR allows an endoplasmic reticulum (ER) homeostasis leading to cell survival and appropriate ECM synthesis. If sustained, activation of the UPR leads to apoptosis. Recently, we have shown that PiT1, mostly described as a phosphate transporter, is expressed in the ER of chondrocytes. To

elucidate its cellular functions and physiological role, we have generated inducible chondrocyte-specific PiT1 knockout mice ($PiT1^{cKO}$) by crossing $PiT1^{lox/lox}$ with $Agc1^{tm(IRES\text{-}creERT2}$ mice and treated by tamoxifen at postnatal day (P) 3 (french ethical approval #02286.01). Histological analysis of humerus sections at P5 revealed the presence of a hypocellular zone in the center of the growth plate due to massive cell death. Analysis of the phenotype at earlier stages showed an upregulation of the UPR-associated pro-apoptotic factor CHOP in PiT1-depleted cells. The ultrastructure of $PiT1^{cKO}$ chondrocytes analysed by electron microscopy showed a massively distended ER, a hallmark feature of ER-stressed cells. *In vitro*, triggering of an ER stress in primary chondrocytes and ATDC5 chondrogenic cells led to a strong induction of *PiT1* expression which was dependent on the UPR transducers $ATF6_N$, ATF4 and XPB1s. Furthermore, PiT1-depleted ATDC5 cells showed an increased sensitivity to ER stress-induced apoptosis as demonstrated by CHOP upregulation. Importantly, overexpression of the wild-type PiT1 or phosphate transport-deficient PiT1 mutant rescued this phenotype, illustrating that the implication of PiT1 in UPR is independent from its phosphate transport function. Our results suggest that under ER stress conditions, PiT1 regulates balance between chondrocyte survival and death. Of importance, the phenotype of $PiT1^{cKO}$ mice is reminiscent of HiF1α and PTEN deletion in cartilage. The mechanistic links between HiF1α/ PTEN pathways and PiT1 are currently being investigated in our lab.

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OP36 (P237)

Circulating Microvesicles from Elderly Donors Modulate Osteogenic Differentiation of Mesenchymal Stem Cells Through the Delivery of microRNAs

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Ageing is a complex process that results in the decline of physiological functions due to accumulation of damage in cells and tissues as well as due to reduced repair capacities. The regenerative power of stem and progenitor cells has been found to decline with age and to be influenced by the systemic environment. In particular, the osteogenic differentiation capacity of mesenchymal stem cells (MSCs) has been shown to decrease with age thereby contributing to decelerated bone formation and the development of osteopenia or osteoporosis. The objective of this study was to identify circulating factors of the aged systemic environment that influence the functionality of adult stem cells. In order to identify such factors, the microRNA content in serum of young versus elderly healthy individuals was analysed using quantitative PCR. Levels of hsa-miR-31-5p were found to be strongly induced in serum of elderly donors. As a possible source senescent endothelial cells that secrete exosomal miR-31-5p were identified in vitro, since it could be shown that exosomal miR-31-5p can be transferred to target cells such as MSCs. Subsequently, exosomes with elevated levels in miR-31-5p were harvested either from senescent endothelial cells or elderly donors, and used for treatment of MSCs prior and during osteogenic differentiation, resulting in slowed differentiation. The effect of the vesicles could be rescued by antagonistic miR-31-5p, and mimicked by delivery of miR-31-5p alone. One of the novel targets of miR-31-5p in this context is the WNT ligand FZD3 whose knock-down leads to a similar inhibition of osteogenic differentiation as miR-31-5p. In conclusion, we could identify a novel mechanism by which circulating microvesicles and their content might impact tissue physiology during ageing. Furthermore these data show that microvesicles might represent a source for biomarkers as well as therapeutic targets in age-related diseases like osteoporosis.

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OP37 (P238)

Circulating microRNAs that are Induced by Osteoporotic Fractures Modulate Osteogenic Differentiation of Mesenchymal Stem Cells

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MicroRNAs (miRNAs) regulate gene expression on a post-transcriptional level and are known to take part in the control of bone formation and bone resorption. In addition, it is known that miRNAs are secreted by many cell types and can transfer "messages" to recipient cells. Thus, circulating miRNAs might not only be useful as surrogate biomarkers for the diagnosis or prognosis of pathological conditions, but could be actively modulating tissue physiology. The objective of this study was to test whether circulating miRNAs that exhibit changes in recent osteoporotic fracture patients could be causally related to bone metabolism. For this purpose an explorative qPCR analysis of 175 miRNAs in serum samples obtained from 7 female patients with recent osteoporotic fractures at the femoral neck, and 7 age-matched controls was performed. Unsupervised cluster analysis revealed a high discriminatory power of the top 10 circulating miRNAs for patients with recent oste-

oporotic fractures. In total 6 miRNAs, miR-10a-5p, miR-10b-5p, miR-133b, miR-22-3p, miR-328-3p, and let-7g-5p exhibited significantly different serum levels in response to fracture (multiple testing adjusted p-value < 0.05). These miRNAs were subsequently analysed in a validation cohort comprising 23 patients (11 control, 12 fracture), which confirmed significant regulation for miR-22-3p, miR-328-3p, and let-7g-5p. A set of these and of other circulating miRNAs previously reported in the context of osteoporosis were subsequently tested for their effects on osteogenic differentiation of human mesenchymal stem cells (MSCs) *in vitro*. The results show that 5 out of 7 tested miRNAs could modulate osteogenic differentiation *in vitro*. Overall, these data suggest that levels of specific circulating miRNAs change in the context of recent osteoporotic fractures and that such perturbations of "normal" levels might affect bone metabolism or bone healing processes.

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OP38 (P390)

Development of a Lentiviral Vector to Express RANKL in Mesenchymal Stem Cells for the Therapy of RANKL-Dependent Osteopetrosis

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Autosomal Recessive Osteopetrosis (ARO) is a rare bone disease characterised by an increase in bone density due to the failure of bone resorption by impaired osteoclast development or function. The only therapy is haematopoietic stem cell transplantation, which, however, is not effective in osteoclast-poor RANKL-dependent ARO, since in bone RANKL is produced mainly by stromal cells. On the other hand, mesenchymal stem cells (MSCs) transplantation (MSCT) could represent a possible effective therapy. To verify this hypothesis, we established bone marrow derived MSCs (BM-MSCs) lines from the Rankl$^{-/-}$ mouse model, which recapitulates the human disease, and we developed a third generation lentiviral vector expressing human soluble RANKL (hsRANKL) for their correction. This vector carries hsRANKL under the CMV promoter and GFP under the hPGK promoter. The lentivirus production was performed by calcium phosphate transfection in HEK293T cells with the pMDL-g/pRRE, pMD2-VSVg, pRSV-Rev plasmids. In order to evaluate transduction efficiency, the produced vector was tested by transducing HEK293T cells at different multiplicity of infection (MOI). Fluorescence microscopy and FACS analysis showed about 100% GFP^{+} cells, while hsRANKL production, assessed by western blot and ELISA on the culture supernatant, increased proportionally to the MOI (ranging from 1 to 100) and was stable over time. However, the higher the MOI (50 and 100 MOI), the higher the cytotoxicity observed. Based on these data, we performed a lentiviral hsRANKL transduction in Rankl$^{-/-}$ BM-MSCs at 20 and 50 MOI, to define the optimal transduction conditions. After transduction 99.5% of MSC were GFP^{+}. While in Rankl$^{-/-}$ control cells the cytokine was not detected, in corrected cells RANKL production and secretion was measurable and comparable with sRANKL levels in wild type (WT) mouse and human BM-MSCs. We are currently testing the transduced MSCs by *in vitro* functional assays; then we will use them in Rankl$^{-/-}$ mice.

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ARTHRITIS AND OTHER JOINT DISEASES: TRANSLATIONAL AND CLINICAL

P1

Stem Cell Therapy Evaluation *In Vivo* using a Combined Iron Oxide Nanoparticle and MRI Approach During Antigen Induced Arthritis

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Background: To detect and track super paramagnetic iron oxide nanoparticles (SPIONs) labelled human mesenchymal stem cells (hMSCs) *in vivo* during the course of antigen induced arthritis (AIA) rat model.
Methods: hMSCs were cultured and labelled *in vitro* using 0.2 mg Fe/ml SPIONs for 24 hours and non-labelled hMSCs were used as a control. After verifying hMSCs SPION labelling and cell viability, 500,000 SPION-labelled and control hMSCs as well as 9 µg SPIONs were injected intra-articularly (i.a.) on day 2 post-AIA induction. The animals were scanned on days 3, 6 and 10 using Siemens 3T clinical scanner with a 4 cm loop coil. The following MR sequences were used: T2 STIR, VIBE and UTE to visualise oedema, SPIONs and SPIONs as a positive contrast respectively. At the end of the experiment the animals were sacrificed, the knees isolated and processed for histology. The sections were Prussian blue stained for SPION visualisation and immunostained for CD44 as a hMSC marker consecutively.
Results: A strong SPION signal was detected on MR images 24 hours after the i.a. injection of SPION labelled hMSCs on both VIBE and T2 STIR sequences and could be detected up to day 10. This signal significantly changed between the different timepoints and its evolution pattern was different from that of SPIONs alone. No signal was found when non-labelled hMSCs or vehicle alone were injected. Furthermore, the signal caused by SPIONs alone had a specific distribution in the synovium which was significantly different from that of the SPION-labelled hMSCs. Co-localisation of Prussian blue particles and CD44 positive cells on histological sections confirmed a persisting uptake of the SPIONs by hMSCs.
Conclusion: Our findings show the feasibility of *in vivo* tracking of SPION labelled hMSCs in AIA. This protocol can be used to assess the outcome of regenerative therapy in arthritis.
Disclosure: The authors declared no competing interests. The research leading to these results has received funding from the European Union Seventh Framework Programme NMP-2008-4.0-1, GRANT AGREEMENT No 228929 (Project NanoDiaRA).

P2

Severity of Diabetes Mellitus and Risk of Total Hip or Knee Replacement: a Population Based Case-Control Study

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Background: The aim of this study was to evaluate the risk of hip or knee replacement, as a proxy for severe osteoarthritis (OA), in patients with diabetes mellitus (DM). We additionally evaluated the risk of total joint replacement (TJR) with various proxies for increased DM severity.
Methods: We performed a population based case-control study using the Clinical Practice Research Datalink (CPRD). Cases (n=94,609) were defined as patients >18 years who had undergone TJR between 2000 and 2012. Controls were matched by age, gender and general practitioner (GP) practice. Conditional logistic regression was used to estimate the risk of total knee (TKR) and total hip replacement (THR) surgery associated with use of antidiabetic drugs (ADs). We additionally stratified current AD users by proxies for OA severity.
Results: Current AD use was significantly associated with a lower risk of TKR (OR=0.86 [95% CI=0.78-0.94]) and THR

(OR=0.90 [95% CI=0.82-0.99]) compared with patients not using ADs. Moreover, risk of TKR and THR was decreased with increasing HbA_{1c}.
Conclusions: Contrary to previous research, our study suggests that DM patients are less likely to suffer from severe OA as compared to non-users. Moreover, risk of severe OA necessitating TJR decreases with increasing DM severity, based on HbA_{1c} values. This is possibly due to dissimilarities in methodology, a decrease in eligibility for surgery, or variability of OA phenotypes.
Disclosure: The authors declared no competing interests.

P3
The Effects of Denosumab Treatment on Bone Mineral Density, Structural Damage in Patients with Osteoporosis and Rheumatoid Arthritis
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Background: RANK-ligand is essential for osteoclast development, activation, and survival. The aim of this study was to evaluate the effects of denosumab (monoclonal antibody that binds RANKL) on BMD, structural damage in patients with rheumatoid arthritis (RA) and osteoporosis (OP).
Methods: 29 postmenopausal women with RA and OP received s/c denosumab 60 mg injections every 6 months for 12 months. The primary endpoint was the change from baseline in the Sharp/van der Heijde (SVH) score, X-ray morphometric analysis of deformations in vertebrae (Genant method) and BMD (by dual energy X-ray absorptiometry) at three sites: lumbar spine (L1-L4), hip neck (HN) and distal forearm (DF) at 12 months. The Statistica 6.0 was used.
Results: The mean age was 58,2±7,5 years, the mean duration of RA 19,8±12,0 years. During the study 18 patients (62,1%) continued glucocorticoids (GC). According to X-ray 9(31,0%) patients had the 2nd, 8(27,6%) – the 3rd and 12(41,4%) - the 4th stage of RA. Mean BMD (L1-L4) before\after treatment was 0,796 ± 0,088g/cm² and 0,830 ± 0,088g/cm² ($p<0.001$), at HN was 0,627 ± 0,077g/cm² and 0,635 ± 0,079g/cm² ($p>0,05$), at DF was 0,484 ± 0,100g/cm² and 0,498 ± 0,092g/cm² ($p=0.032$), respectively. The significant increase of BMD was noted both in groups, receiving GC or not. The index of vertebral deformations at lumbar spine did not change: 0,79±0,03, at thoracic site was 0,77±0,04 and 0,76 ± 0,04 ($p>0,05$), respectively. The erosion score and total SVH score were increased after treatment ($p=0.043$): 53.3 ± 53.7 and 54.3 ± 53.8, 156.1 ± 84.7 and 157.3 ± 85.0, respectively. The amount of narrowed cracks did not change significant: 102.7 ± 37.1 and 103.0 ± 37.1.
Conclusions: After 12 months denosumab therapy it was shown the significant increase of BMD in L1-L4 and forearm, the erosion score. The index of vertebral deformations remained stable.
Disclosure: The authors declared no competing interests.

P4
High Prevalence of Autoimmune Disease in Patients with Sternocostoclavicular Hyperostosis and their First Degree Relatives
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Background: Sternocostoclavicular hyperostosis (SCCH) is a rare inflammatory disorder of the axial skeleton of unclear aetiology, characterized by chronic sterile osteomyelitis predominantly involving the sternum, medial end of the clavicles and upper ribs. Familial clustering suggests a genetic predisposition. Typical skin lesions of palmoplantar pustulosis (PPP) are prevalent and other skin, joint and gut manifestations are relatively frequently reported, suggesting a possible autoimmune contribution to the pathogenesis of the disorder. The objective was to determine whether autoimmune diseases are prevalent in SCCH patients and their first degree relatives.
Methods: Patients with an established SCCH diagnosis followed up at our Out-patient Clinic and/or members of the Dutch SCCH Patients' Association. Systematic telephone interviews were conducted in SCCH patients. Annotated pedigrees were constructed. Enquiries mainly concerned any diagnosed autoimmune disease in patients as well as their first degree relatives (parents, siblings and children). Diagnoses were wherever possible verified from patients' hospital records.
Results: Seventy-three patients (86% female, aged 18-80 years, median age 58 years) from 65 families were available for interview, out of approximately 92 known patients. Information about 527 unaffected first degree relatives (52.4% female) was documented; 13 patients (18%) had at least one other SCCH patient among their relatives. 20 patients (27%) and 47 relatives (8.9%) had a well-documented autoimmune disease. The prevalence of autoimmune disease increased to 55% (40/73) in SCCH patients, and to 10.4% (55/527) in relatives, by including PPP as putative autoimmune disease. None of the patients reported a diagnosis of inflammatory bowel disease.
Conclusions: Our data suggest that the prevalence of autoimmune disease is high in patients with SCCH and their first degree relatives. This finding warrants further investigation of the role of autoimmunity in the pathophysiology of the intriguing aberrant response of bone to inflammation observed in SCCH.
Disclosure: The authors declared no competing interests.

P5
"Antiresorptive and Anabolic Bone Therapy has no Benefit to Healing of the Articular Cartilage in Osteoarthritic Rats"
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Osteoarthritis (OA) is a complex disease that affects bony and cartilaginous joint structures. Progression of OA is associated with cartilage deterioration, sclerosis of the subchondral bone and osteophyte formation. The goal of this study was to

investigate whether treatment with antiresorptive (zolendronate) or anabolic therapy (PTH) benefits healing of the articular cartilage in the medial meniscal tear (MMT) model of OA. MMT surgery was performed on male Lewis rats to induce OA. Biomarkers of bone and cartilage metabolism were evaluated in the serum. A dynamic weight bearing (DWB) system was utilised to measure functional capacity of the musculoskeletal system. 3-point bending was used to assess femoral strength. Micro-CT was used to evaluate bone geometry and cartilage morphology. Histomorphometry and histology was used to assess joint morphology. DWB data showed a distinctive difference in weight bearing capacity between sham and MMT rats regardless of treatment. Mechanical testing showed clear differences in cortical bone strength between sham and MMT rats. Contrast mCT and histology data demonstrated degradation of articular cartilage in MMT rats regardless of treatment. Dynamic bone histomorphometry showed increased subchondral bone formation and osteophyte formation in all MMT rats regardless of the treatment paradigm. Results indicate clear insufficiency in the weight bearing capacity of an operated leg. The under-loading of the operated leg caused by pain and mechanical damage to the articular cartilage led to bone loss and weaker cortical bone. Similar degree of cartilage degradation, osteopetrosis and osteophyte formation was seen in all MMT rats. There was no clear benefit of antiresorptive or anabolic bone therapy to the healing process of articular cartilage.
Disclosure: All authors are employed by Pfizer.

P6

Targeted Depletion of Resident Synovial Macrophages in Antigen Induced Arthritis using Liposome Encapsulated Clodronate Reduces Iron Oxide Nanoparticle MRI Signal *In Vivo*

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Encapsulating clodronate in a liposome bilayer (Clod-Lipo) enhances its phagocytosis by macrophages and subsequently its effectiveness in killing the phagocytic cells. In this study, we have employed this technique to compare the effect of eliminating different macrophage/monocyte populations on super paramagnetic iron oxide nanoparticles (SPION) uptake in the knee joint during antigen induced arthritis (AIA). To achieve this goal, rats were injected with Clod-Lipo or its negative control liposome encapsulated PBS (PBS-Lipo) twice before AIA induction either intraperitoneally (i.p.); targeting the spleen macrophages, intravenously (i.v.); targeting mostly monocytes but also reticulocytes in the liver and spleen or intra-articularly (i.a.); targeting synovial macrophages. Five days after AIA induction, the rats received 2.5 mg SPION via i.v. injection. The SPION signal was monitored using VIBE MR sequence on days 6 and 10. The 3D MR signal was quantified using a new segmentation software. At the end of MR scanning, the knee joints were dissected, sectioned and analyzed to confirm the MR results. Comparing the effect of the different injection routes on the SPION signal in the knee joint revealed that only the i.a. injection of Clod-Lipo was effective in reducing that signal on day 6 which almost disappeared by day 10. Injecting Clod-Lipo through the i.v. and i.p. routes had no or little effect on that SPION signal. We also noted a significant decline in intra-articular oedema in the the i.a. Clod-Lipo group. The histological assessment on Prussian blue stained sections depicting SPION clusters also confirmed the MR results where a 65% decrease in the number and area of Prussian blue particles was found between the Clod-Lipo group and the non-treated group and PBS-Lipo groups.
Disclosure: The authors declared no competing interests. The research leading to these results has received funding from the European Union Seventh Framework Programme NMP-2008-4.0-1, GRANT AGREEMENT No 228929 (Project NanoDiaRA).

P7

Use of Thiazolidinediones and the Risk of Elective Hip or Knee Replacement: a Population Based Case-Control Study

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Background: The aim was to determine the risk of total joint replacement (TJR) in patients using thiazolidinediones (TZDs) compared with those not using TZDs.
Methods: A population based case-control study was performed using the Clinical Practice Research Datalink (CPRD). Cases (n=94,609) were defined as patients >18 years of age who had undergone TJR between 2000 and 2012. Controls were matched by age, gender and general practitioner (GP) practice. Conditional logistic regression was used to estimate the risk of total knee (TKR) and total hip replacement (THR) associated with use of TZDs. We additionally evaluated risk of TJR in current TZD users compared with DM patients using other ADs. In order to determine a dose effect relationship, we also stratified TZD users by total number of prescriptions prior to surgery.
Results: There is no difference in risk of TKR (OR=1.11 [95% CI=0.95-1.29]) or THR (OR=0.87 [95% CI=0.74-1.02])

between TZD users and patients not using TZDs. Furthermore, there is no difference in risk of TKR (OR=1.03 [95% CI=0.88-1.22]) and THR (OR=0.90 [95% CI=0.75-1.08]) when TZD users are compared with other AD users. Finally, we did not find a dose response effect with increasing number of prescriptions.
***Conclusion*:** Despite promising results from *in vivo* studies, this study did not find any evidence for an anti-arthritic effect of TZDs.
Disclosure: The authors declared no competing interests.

P8

TRACP Regulates the Phosphorylation of Osteopontin in Synovial Fluid of Osteoarthritis and Rheumatoid Arthritis Patients

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Background: Osteopontin is a proinflammatory protein which production is shown to increase in rheumatoid arthritis (RA) and osteoarthritis (OA). OPN's cytokine properties are dependent on phosphorylation, which is controlled by extracellular tartrate-resistant acid phosphatase (TRACP), also a biomarker for RA. Our objective was to assess the phosphorylation status of OPN in RA and OA, and its correlation with TRACP levels.
Methods: Synovial fluid was obtained from 16 RA patients (8 seropositive and 8 seronegative) and four OA patients. Western blot method was used to analyse OPN's phosphorylation and TRACP levels in synovia. The protein membranes were exposed to chemiluminescence films and band optical densities were quantified. Immunohistochemistry was done to find TRACP secreting cells in synovial tissue.
Results: Immunoblotting showed the presence of phosphorylated OPN and TRACP in both RA and OA synovia. Full length OPN was more heavily phosphorylated in RA (seropositive OD 0.200±0.018) than in OA (OD 0.153±0.017) (P=0.011). Thrombin cleaved C-terminal end of OPN was also more phosphorylated in RA (P=0.003). TRACP correlated negatively with phospho-OPN. RA patients had less TRACP in their synovia (seropositive OD 0.287±0.071) than OA patients (OD 0.398±0.076) (P=0.015). TRACP secreting cells were found all along the synovial epithelium with immunohistochemistry.
Conclusion: Phosphorylated OPN in synovia leads to an increased macrophage activation and inflammation in RA patient joints when compared with OA patient joints. Phospho-OPN is also shown to increase the production of cartilage degrading enzymes in chondrocytes, and bone resorption in osteoclasts. Although it is shown that TRACP production increases in RA when compared with healthy subjects, the enzyme levels are not high enough to dephosphorylate enough OPN as in OA levels of phospho-OPN are lower.

Disclosure: The authors declared no competing interests.

P9

The Influence of Steroids Therapy on Bone Tissue in Patients with Rheumatoid Arthritis

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Background: The aim was to receive data on bone mineral density (BMD) and erosive and destructive changes in hands and feet rheumatoid arthritis(RA) patients with/without glucocorticoids(GC) therapy.
Methods: In this research it was included 152 women with RA, age 20-67 years. The BMD was assessed in all patients by dual energy X-ray absorptiometry (DXA) at three sites: lumbar spine (L1-L4), hip neck (HN) and distal part of forearm (DF) of nondominant hand. The X-ray morphometric analysis of vertebral bodies deformations was used with Genant method. The assessment of radiological signs of RA progressing was used with Sharp/van der Heijde method in 134 years.
Results: The mean age of patients included in this research was 50,3±12,1 years, the age at the time of the beginning of RA was 36,9±12,1 of years. We distributed patients in two groups: the group 1 received GC therapy - 101(67%) patients, the group 2 didn't receive - 51(33%) patients. The age of patients was comparable; RA duration - 14,5±9,7 vs 9,4±8,3 years respectively (p<0,05). Osteoporosis (OP)at least in one of analyzed sites was revealed in 72(72%) vs 14(27%) patients, respectively (p<0,05). BMD at DF was 0,887±0,147 g/cm2 vs 0,960±0,166 g/cm2 in groups 1 and 2 respectively (p<0,05), at HN it was 0,688±0,122 g/cm2 vs 0,739±0,125 g/cm2 respectively(p<0,05). The fractures of peripheral skeleton were in group1 in 40(40%), in group2 17(33%) patients. The index of erosion in group 1 was 48,9±53,9 points, in group 2 - 19,6±29,9 points(p<0,05), the amount of narrowed cracks of 99,6±38,4 vs 72,6±36,5 points respectively(p<0,05), the total Sharp/van der Heijde score was 148,8±83,9 vs 92,2±60,9 of points, respectively(p<0,05).
Conclusions: GC had a negative impact on BMD at HN and DF that promoted the development of OP. The reception of GC didn't stabilise the indices of erosive and destructive changes in hands and feet. Fractures of peripheral bones and vertebras were observed with identical frequency regardless of GC therapy.
Disclosure: The authors declared no competing interests.

P10

Chemokine Receptor Profile of Osteoclast Progenitor Cells in Patients with Rheumatoid Arthritis

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Background: Rheumatoid arthritis (RA) is a chronic joint disease marked by persistent inflammation and osteodestruction. The mechanisms leading to joint destruction involve infiltration of osteoclasts, multinucleated cells derived from

monocyte/macrophage lineage. Human osteoclast progenitors (OCPs) are contained among peripheral blood monocytes at low frequency even in healthy subjects. OCPs exhibit chemotaxis and, furthermore, synovial compartment of RA patients highly expresses different chemokines. The aim of our study was to define these chemotactic signals by analysing expression of several chemokine receptors on OCPs in the peripheral blood, the levels of their respective ligands in serum and synovial fluid of RA patients and to assess differentiation potential of isolated OCPs.

Methods: Mononuclear cells were separated from peripheral blood of healthy controls and RA patients. The phenotype of OCPs (CD3-CD19-CD56-CD11b+CD14+) was determined using flow cytometry for the following chemokine receptors: C5AR1, CCR1, CCR2, CCR4, CXCR4. Chemokine ligand concentrations (MIP-1α/CCL3, MIP-1β/CCL4, MCP-1/CCL2, RANTES/CCL5) were measured in serum and synovial fluid of RA patients using flow cytometry bead based array. OCPs were sorted and cultured with M-CSF and RANKL. After two weeks, the cells were stained for TRAP enzyme and positive, mature, osteoclasts were counted.

Results: Human peripheral blood OCPs similarly expressed chemokine receptors CCR1, CCR2, CCR4 and CXCR4 in RA and healthy subjects. However, MCP-1/CCL2, MIP1a/CCL3 and MIP1b/CCL4 concentrations were significantly higher in synovial fluid, as well as CCL2 and CCL4 in serum. Cell culture revealed no significant differences in mature osteoclast count between RA and control group.

Conclusions: Although OCPs in RA have a differentiation potential similar to controls, levels of several chemokines are upregulated, indicating a possible chemotactic mechanism of OCP migration to affected joints. These results may help to reveal a migration mechanism of OCPs specifically associated with RA in order to develop more efficient therapeutic approaches.

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P11

Effect of TNF Blocking Therapy on Osteoclasts from Ankylosing Spondylitis Patients

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Ankylosing spondylitis (AS) is a chronic inflammatory rheumatic disease of the axial skeleton, characterised by systemic osteoporosis along with new local bone formation. Previous studies have shown that serum levels of TNF, IL-6 and IL-17 are increased in AS patients and may be implicated in the development of secondary osteoporosis, since these cytokines are able to induce osteoclast (OC) differentiation and bone resorption. In this work we aimed to assess the effects of TNF-blocking therapy in the systemic inflammatory environment of AS patients as well as in OC differentiation and activity. Patients with AS starting TNF-blocking therapy were recruited for this study and blood was collected at baseline and 6 months after the first treatment. We performed ELISAs in the serum of patients to assess cytokine levels. We also characterised RANKL surface expression in circulating leukocytes and monocyte subpopulation frequency and phenotype by flow cytometry. We cultured circulating monocytes from AS patients, before and after therapy under osteoclastogenic conditions and we performed TRAP staining and resorption pit assay. We found no differences (before and after treatment) in any of the circulating monocytes' subpopulations regarding frequency or cell death. Phenotype analysis only revealed differences in the classical monocyte subpopulation where there was a significant decrease in HLA-DR expression after treatment. The frequency of RANKL positive B lymphocytes was reduced after treatment. No changes were observed on RANKL expression in neutrophils or T lymphocytes after treatment. Before TNF-blocking treatment AS patients have increased levels of pro-inflammatory cytokines when compared with healthy subjects. After TNF-blocking therapy IL-17, TGF-β and osteoprotegerin were significantly decreased. Interestingly, we observed that after TNF-blocking therapy differentiated OCs have increased resorption activity. Our results suggest that in AS patients there might be a paradoxical effect of TNF blocking therapy that induces OC activity.

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P12

Identification of Antithrombotic Drugs Related to Total Joint Replacement using Anonymised Free Text Notes

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Background: Intensive antithrombotic treatment, predominantly dispensed at the hospital, is recommended to prevent

venous thromboembolic events after total joint replacement (TJR). Unfortunately, hospital prescription data is often lacking in general practitioner databases, thereby limiting the applicability of these rich databases to study long-term effects in real practice. Un-coded anonymised free text from hospital discharge letters may be used to collect additional information.
Objective: To design and test a method to extract additional information on anticoagulation therapy in patients undergoing TJR from anonymised free text notes in the Clinical Practice Research Datalink (CPRD).
Methods: Anticoagulant drug use related to total hip (THR) or knee replacement (TKR) was identified using both anonymised free text and prescription data. Internal validity of our newly designed method was determined by calculating positive predictive values (PPVs) of hits for predefined keywords in a random sample of anonymised free text notes. In order to determine potential detection bias, TJR patient characteristics were compared with regard to their status of exposure to antithrombotics.
Results: PPVs ranging between 97% - 99% for NOAC or LMWH exposure related to TJR were obtained with our method. Our algorithm increased detection rates by 57%, yielding a total proportion of 18.5% of all THR and 18.6% of all TKR surgeries. Identified users of NOACs and LMWHs were largely similar with regards to age, sex, lifestyle, disease and drug history compared with patients without identified drug use.
Conclusion: We have developed a useful method to identify additional exposure to NOACs or LMWHs with TJR surgery.
Disclosure: The authors declared no competing interests.

P13
The Analysis of the Course of Rheumatoid Arthritis Depending on the Climatic and Geographical Zones of Uzbekistan
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Background: Our aim was to retrospectively analyse the situation and structure of RA in different regions of Uzbekistan.
Method: For the analysis, extracts of clinical records of RA patients were used. Analysis was conducted and regions were divided conditionally into three geographical zones: zone I - Namangan, zone II - Surkhandarya and zone III - Khorezm region.
Results: Course of RA in three various climatic and geographical zones have a certain distinction. So, in zone III, indicators such as the tendency to progression of the disease over the past 3 years was dominated with figure 83.4%; while the frequency of cases with a need for hospitalisation in one year was 67% and there was a high rate on existence of seropositive results with the point of 75%. In zone II manifestation of the disease in the majority of patients had an earlier age - 48% of all cases, changes in reproductive system, i.e. the women with RA within a year most often addressed with the problems associated with a menstrual a cycle violations– 65%, while this figure was only 17% and 28% for zone III and zone I, accordingly. In zone II - in 36.4% of women spontaneous abortion occurred, whereas 26.7% of female patients registered with secondary amenorrhea, when in 73.3% of women a various types of menstrual irregularities were the case. In contrast, zone I distinguished itself by a relatively low (positive) performance compared with other zones. However, in this zone, at the majority of patients (59%) the manifestation of the disease was characterised by a gradual deterioration of a patient's condition.
Conclusion: The retrospective analysis demonstrated that clinical and epidemiological parameters of RA are different in the three zones of Uzbekistan, which does not exclude the probability of the impact of climatic and geographical factors on the course of a disease.
Disclosure: The authors declared no competing interests.

BONE BIOMECHANICS AND QUALITY

P14 (OP23)

P15 (OP29)

P16 (OP1)

P17
Differences of Acoustic Elastic Properties Between Periosteal and Endosteal Bone are Abolished in Brtl/+ Mice
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The Brtl/+ mouse is a heterozygous knock-in model for OI type-IV with a Gly349Cys substitution in one *COL1A1* allele. While mechanical alteration at whole bone level in these mice is established, little is known about the contribution of bone material properties to OI bone fragility and brittleness. Transverse femoral midshaft sections (30µm thickness) of 2-month old mice (wild-type n=6; Brtl/+ n=6) were studied. Elastic properties were assessed using time-of-flight scanning acoustic microscopy in combination with quantitative backscattered electron imaging (qBEI). Sound velocity (2µm pixel, 0.125ns time-resolution) and calcium content-derived material density maps were combined to extract dynamic elastic moduli (E-modulus) maps. The measurements were focused on relatively homogenous bone avoiding the third trochanter and excluding regions rich in vascular canals. We focused on bone matrix deposited from endosteal and periosteal surfaces, respectively designated as endosteal region (ER) and periosteal region (PR), and further excluded regions with residual cartilage and woven bone. The analysis

revealed that wild-type material density (+2.1%), sound velocity (+6%) and E-modulus (+15%) were all significantly greater in PR than in ER. In contrast, Brtl/+ mice had significantly increased ER sound velocity (+9.4%) and E-modulus (+22%) compared with wild-type ER. This change in ER properties of Brtl/+ bone abolished the normal differences between E-modulus in periosteal and endosteal bone, despite the lower material density (-1.8%) of Brtl/+ ER compared with PR. Our findings indicate that the bone matrix formed from Brtl/+ endosteal osteoblasts has a higher E-modulus than expected despite material density values similar to wild-type. Elastic properties of mineralised collagen are known to depend strongly on fiber orientation, and we found that the lamellar organisation of Brtl/+ bone appeared consistently less distinct under polarized light than in WT femora. Therefore, the unexpected mechanical properties of Brtl/+ bone are most likely linked to alterations in collagenous fibril arrangement.

Disclosure: The authors declared no competing interests.

P18

Bisphosphonate Treatment During an Initial Unloading Period Provides Beneficial Effects to Mechanical and Densitometric Properties of Bone for a Second Unloading

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Because anti-resorptive effects of bisphosphonates (BP) are known to persist following treatment cessation, we hypothesised that beneficial effects of BP treatment given for an initial unloading exposure only, and then withdrawn, would extend to a second unloading exposure. The adult hindlimb unloaded (HU) rat model was used to simulate two successive spaceflight missions, and two BPs were compared, alendronate (AL) and zoledronic acid (ZA). Adult Sprague-Dawley male rats (6 mo.) were block assigned to ageing control (AC) and HU groups by body weight. HU animals were exposed to 28d of HU, followed by 56d of recovery, and then a 2nd 28d HU exposure. Subsets of HU animals were administered AL (HU+A), ZA (HU+Z), or nothing (HUC) for the initial 28d of HU only. AL (2.4 µg/kg) was injected 3x/week for 5 weeks, starting the week before the first HU. ZA (60 µg/kg) was injected in a single dose prior to the first HU. *In vivo* pQCT scans were taken of the proximal tibia metaphysis (PTM) at baseline (BL) and every 28 days. *Ex vivo* analyses, conducted after the end of the study following the 2nd HU period, included micro-CT of the PTM and both pQCT and mechanical testing of the femoral neck (FN). For *in vivo* pQCT results, AL prevented losses for the 1st HU, as both total bone mineral content (BMC) and density (vBMD) for HU+A were not different from baseline (BL) or AC at day 28. In contrast, ZA induced absolute gains in both total BMC and vBMD, with HU+Z significantly higher after the 1st HU compared with BL, AC, and HUC. The efficacy of ZA continued throughout recovery and into the 2nd HU, with total vBMD unchanged for HU+Z and significantly higher than BL (+14.2%), AC (+13.7%), HUC (+16.2%), and HU+A (+10.7%). For HU+A, total vBMD was not different from AC at the start and end of the 2nd HU. For *ex vivo* results, bone volume fraction (BV/TV) was significantly higher for HU+Z compared to BL, AC, HUC, and HU+A; whereas, BV/TV values were not different for HU+A. Trabecular thickness (Tb.Th) results were similar, with ZA more beneficial than AL. For *ex vivo* mechanical testing, the femoral neck (FN) maximum fracture load was significantly higher for HU+Z compared to every other group, with no other statistical differences. The primary effects on pQCT results at the FN were significantly higher trabecular BMD for all groups compared with HU, with ZA the highest (23%; v. 16% for AL).

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P19

Fast Estimation of Colles' Fracture Load of the Distal Radius by Homogenised Finite Element Analysis Based on State of the Art High Resolution Peripheral Computed Tomography

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Osteoporosis is a skeletal disorder characterised by compromised bone strength and increased fracture risk. Fractures of the distal radius (Colles' fractures) occur earlier in life than other osteoporotic fractures. High resolution peripheral quantitative computed tomography (HR-pQCT) enables the *in-vivo* assessment not only of local bone mineral density (BMD) but also of 3D morphometric indices such as trabecular orientation (fabric). The aim of the current work was to validate experimentally a fast patient-specific homogenised finite element (hFE) model of the human distal radius and to develop a fully automated diagnostic tool for the *in-vivo* prediction of Colles' fracture load for the last generation HR-pQCT scanner (XtremeCT II, SCANCO Medical). For this purpose, 12 pairs of cadaveric human forearms (mean age = 77.2 ± 8.5 years) were scanned intact using the high (61 µm) and the low (82 µm) resolution protocols to replicate the *in-vivo* scanning conditions. The 20 mm most distal radius sections were dissected out of the forearms and scanned at a resolution of 16.4 µm on a µCT 100 (SCANCO Medical) used to calibrate BMD and fabric obtained from the XtremeCT II reconstructions. These radial sections were tested in compression to assess failure loads. The tests were simulated

by non-linear hFE and linear microFE analyses based on XtremeCT II images with *a priori* determined material constants. The computed fracture loads were strongly correlated with the experiments: R^2=0.95 and R^2=0.94 for the hFE and R^2=0.93 and R^2=0.94 for the microFE models based on the high and low resolution protocols, respectively. Computation of fracture load with hFE was around 3 times faster than with microFE for a similar accuracy. This study delivers an extensive validation for the *in-vivo* use of an accurate and fast hFE diagnostic tool to improve fracture risk, prediction, and treatment follow-up of patients with osteoporosis.
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P20
Effects of Stable Incretin Mimetic on Cortical Bone Micromorphology and Strength in a Murine Model of Type 1 Diabetes Mellitus
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Background: Type 1 diabetes mellitus is associated with a high risk for bone fractures. Although bone mass is reduced, bone quality is also dramatically altered in this disorder. However, recent evidence suggests a beneficial role of glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide 1 (GLP-1) pathways on bone quality. The aims of the present study were to conduct a comprehensive investigation of cortical bone strength at the organ and tissue level and to ascertain whether enzyme-resistant GIP or GLP-1 mimetics could be beneficial for treating bone fragility in type 1 diabetes mellitus.
Methods: Streptozotocin-injected mice were used as a model of type 1 diabetes mellitus. Control and streptozotocin-injected animals were treated with an enzymatic-resistant GIP peptide ([D-Ala^2]GIP) or with the GLP-1 mimetic, liraglutide. Bone quality was assessed at the organ and tissue level by microCT, qXRI, 3-point bending, qBEI, nanoindentation and Fourier-transform infrared microspectroscopy. Non-parametric Mann-Whitney U-test was used to compare differences between groups.
Results: Cortical bone strength was dramatically altered in STZ-injected mice as compared with control animals. [D-Ala^2]GIP and liraglutide treatment did not significantly improve whole bone strength in STZ mice, except for post-yield displacement that was substantially higher in both treatment groups. Cortical microarchitecture was also severely impacted in the STZ-injected mice with an 18 % reduction in cortical thickness. [D-Ala^2]GIP and liraglutide treatment did not significantly increase cortical thickness. Alterations of tissue material properties were evidenced in STZ-injected mice with significant reductions in maximum force, hardness, indentation modulus and dissipated energy. Treatment with [D-Ala^2]GIP or liraglutide significantly improved these parameters. STZ mice also presented with a reduction in collagen integrity that was restored by treatment with [D-Ala^2]GIP or liraglutide.
Conclusions: Treatment of STZ-diabetic mice with [D-Ala^2]GIP or liraglutide significantly improves the quality of bone matrix. Further studies are required to further elucidate the molecular mechanisms involved and to validate whether these findings can be translated to the human setting.
Disclosure: The authors declared no competing interests.

P21
DXA-Based Finite Element Models for Prediction of Femoral Strength: Validation with Experiments and Comparison with aBMD and QCT-Based Finite Element Models
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Femoral fractures cause excess morbidity, disability and mortality and are a major health problem, which is likely to be aggravated by the ageing of population. Areal bone mineral density (aBMD) measured with dual energy X-ray absorptiometry (DXA) is the gold standard clinical measure to evaluate fracture risk *in vivo*. Three-dimensional measurements can be performed with Quantitative Computed Tomography (QCT), but inducing higher radiation dose. DXA-based and QCT-based Finite Element (FE) analysis have been developed in recent years in order to improve the prediction of femoral strength and therefore of fracture risk. However, these models should be validated with accurate experiments *in vitro* before a clinical application. The aim of this study was to evaluate the ability of aBMD, DXA-based and QCT-based FE models in predicting the femoral strength in two loading conditions. Thirty-six pairs of human femora were dissected and scanned with DXA and QCT. aBMD was measured in different sites of the proximal femur. For each pair, one femur was tested in one-legged stance configuration (STANCE) and the contralateral one in a backward sideways fall configuration (FALL). Nonlinear QCT-based FE and linear DXA-based FE models were generated reproducing the same loading configurations imposed in the experiments. For experiments and models, the femur failure loads were computed and compared. For the FALL configuration the best predictors of bone strength was the QCT-based FE (R^2=0.85), followed by femoral neck aBMD (R^2=0.80) and DXA-based FE (R^2=0.76). For the STANCE configuration the best predictor was QCT-based FE (R^2=0.80), which was similar to the DXA-based FE (R^2=0.79), both were better than the femoral neck aBMD (R^2=0.66). While QCT-based FE models have been found to be superior to both aBMD and DXA-based FE models in both configurations, the DXA-based FE models have shown good improvement for the STANCE configuration compared with femoral neck aBMD.
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P22

Coalignment between the Canaliculi of the Osteocyte Network and the Collagen Fibre Orientation in Human Osteons

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Osteocytes are embedded in the mineralised bone matrix and are connected with each other via a dense network of canaliculi. The osteocyte network is thought to control bone remodelling and to contribute to mineral homeostasis via the process of osteocytic osteolysis. Our investigations focus on how the osteocyte lacuno-canalicular network (OLCN) is orientated, specifically in relation to the concentric lamellae within human osteons. Four samples of cortical bone from femora of middle-aged healthy women were stained with rhodamine allowing to three-dimensionally image the network within 10 osteons in each sample using confocal laser scanning microscopy.[1] The image data of the OLCN was skeletonised rendering the network topology. The lamellar structure was obtained using the second harmonic generated (SHG) signal of collagen, which allowed defining dark lamellae (collagen orientation predominantly perpendicular to the image plane) and bright lamellae. Most of the network is oriented radially towards the centre of the osteon. More quantitatively, 64±1% of the canalicular length has an angle smaller than 30° to the direction towards the osteon center, while the lateral network - defined by an orientation angle larger than 60° - comprises 16±1%. Within dark lamellae most of this lateral network is parallel to the Haversian canal, and the orientation of the canaliculi twists when moving along the direction of bone deposition across a bright and dark lamella. The lateral network can, therefore, be described by a twisted plywood model being coaligned with the orientation of the collagen matrix. However, our data indicate additional structural changes in the network alignment between bright and dark lamellae. The results of our investigation agree with the hypothesis that dendritic cell processes are involved in the alignment of the collagen matrix during osteoid formation.

Disclosure: The authors declared no competing interests.

Reference

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P23

Prediction of Incident Hip Fracture with Femoral Strength Assessed by Finite Element Analysis of DXA Scans in the Osteoporotic Fractures in Men (MrOS) Study

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Bone fractures only when it is loaded beyond its strength. The purpose of this study was to determine the association of femoral strength, as estimated by finite element (FE) analysis of DXA scans, with incident hip fracture. This prospective case-cohort study included a random sample of 500 men and 170 incident hip fracture cases (16 in the random sample) during a mean±SD follow-up of 7.7±2.2 yrs from the MrOS study (n=5957 community-dwelling men 65 yr of age). We analyzed the baseline DXA scans of the hip using a validated plane-stress, linear-elastic FE model of the proximal femur and derived the femoral strength during a sideways fall. We derived an estimated strength intervention threshold of 4263 N corresponding to the FN BMD T-score = -2.5. Cox regression accounting for the case-cohort design assessed the association of the femoral strength with hip fracture. Compared with the non-fracture group, the intra- and extra-capsular fracture (IC and EC) groups had significantly ($p<0.0001$) lower FN BMD (0.79±0.12 v. IC 0.67±0.11 & EC 0.65±0.10 g/cm^2), TR BMD (0.76±0.12 v. 0.68±0.11 & 0.62±0.10) and TH BMD (0.96±0.13 v. 0.84±0.13 & 0.79±0.11) as well as FE strength (5704±1175 v. 4658±935 & 4726±1048 N). The FE strength was significantly ($P<0.05$) associated with both IC and EC fractures, the age-BMI-adjusted hazard ratio (HR) per SD decrease of the FE strength was 3.49 (95% CI 2.30-5.31) and 3.25 (1.92-5.52) for IC and EC fracture, respectively. The association was still significant ($p<0.05$) for IC fractures after further adjustment for FN, TR and TH BMDs, the HR being 1.93 (1.17-3.20), 3.01 (1.92-4.73) and 2.41 (95% CI 1.53-3.79) respectively. The association with IC fracture was as strong as FN BMD (Harrell's C index for the strength 0.79 v. FN BMD 0.79) and stronger than TR and TH BMDs (0.76 and 0.78). Sensitivities (specificities) for IC fracture prediction were higher with the strength threshold than the FN BMD T-score threshold: 0.36 (0.92) v. 0.29 (0.96), whereas using either of both thresholds improved prediction further (sensitivity 0.45, specificity 0.90). This is the first time that an estimate of strength from DXA has been used to predict hip fracture in men and the results suggest that the strength estimate provides predictive ability for IC fracture in addition to hip BMD but not for EC fracture in older men.

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P24

Endothelial-Osteoblast-Osteoclast Liaison under Unloading Conditions Negatively Affects Bone Homeostasis In Vitro and In Vivo via Lipocalin 2 and Nitric Oxide Synthase 2 Pathways

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Mechanical unloading negatively affects the skeletal homeostasis. Endothelial cells (ECs) are vital bone regulators and sensitive to mechanical stimuli. We hypothesised that ECs are implicated in the control of bone metabolism during unloading. We treated mouse primary osteoblasts with Conditioned Medium from ECs subjected to MicroGravity (MG-EC-CM), demonstrating its ability to increase osteoblast proliferation (+1.5-fold;p=0.003), and decrease Alkaline Phosphatase (ALP) activity (-53%;p<0.001) and matrix nodule formation (-66%;p=0.010) compared with unit gravity EC-CM. MG-EC-CM increased expression of osteoblast RANKL and osteoclastogenesis in osteoblast-bone marrow mononuclear cell co-cultures (+56%;p<0.001). Furthermore, it induced the expression of the osteoblast de-differentiating factor, Lipocalin 2 (Lcn2) (+48-fold;p<0.001), whose silencing recovered osteoblast ALP activity (+52%;p=0.024), decreased RANKL expression and reduced osteoclast formation (-38%;p=0.004), with no effect on osteoblast proliferation. MG-EC-CM enhanced osteoblast NO-Synthase 2 (NOS2) and CycloOXygenase 2 (COX2) expression. Inhibition of NOS2 or NO signalling reduced osteoblast proliferation and rescued ALP activity (+38%;p=0.004). Nuclear translocation of the Lcn2/NOS2 transcription factor, NF-kB, was observed in MG-EC-CM-treated osteoblasts and MG-ECs, alongside a high expression of the NF-kB activator, IL-1β (+96-fold;p=0.002), with NF-κB inhibition reducing osteoblast proliferation and rescuing ALP. Lcn2 (+22-fold) and NOS2 (+12-fold) were also incremented in *ex-vivo* calvaria cultured with MG-EC-CM, and *in-vivo* tibias and calvaria (p=0.021) injected with MG-EC-CM. Furthermore, the tibias of *in-vivo* models of mechanical unloading, including tail-suspended mice and mice treated with botulin A toxin to induce transient muscle paralysis, which featured decreased bone mass, showed increased expression of IL-1β (+5.8-fold;p=0.004), Lcn2 (+10-fold;p=0.002) and NOS2 (+23-fold;p=0.001), suggesting their involvement in the *in-vivo* EC-osteoblast cross-talk. We conclude that, through IL-1β, MG-EC-CM induces NSO2 and Lcn2 in osteoblasts. By means of its downstream NO/COX2 pathway, NOS2 increases osteoblast proliferation, while Lcn2 impairs differentiation and enhances RANKL expression and osteoclastogenesis. Targeting this EC-osteoblast-osteoclast regulatory loop could help improve the bone phenotype in unloading conditions.

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P25

Influence of Obesity on Bone Tissue of Aged *Rattus Norvegicus Albinus* Submitted to Tail Suspension and Resistance Exercise

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In this study, we evaluated the effects that obesity induced by sucrose intake provides in bone tissue of aged male Wistar rats in two opposite situations: in extreme inactivity, and performing strength exercises. Physical inactivity was induced by tail suspension, simulating situations of little or no load on the hindlimbs and the exercises were performed on adapted ladder. 54 male rats with 16 months old were studied. Upon completion 13 months old, 27 rats were randomly selected to receive sucrose-rich diet for 3 months. After this period, they were randomised into three groups: 9 were the Sucrose group (Sa), 9 Sucrose Suspended group (SaSu) and 9 Sucrose Exercise group (SAEx). The remaining 27 animals which didn't received the sucrose-rich diet were randomly assigned at the end of these 16 months, forming 3 more groups: 9 animals were used as controls (C), 9 Suspended group (Su), and 9 Exercise group (Ex). Analyses were made with densitometer to measure bone mineral content and areal bone mineral density in the femurs and tibias; Mechanical tests of compression of the femoral head, tibial compression and compression of the cortical bone and Immunohistochemical analysis with markings for OCN and TRAP. The results showed obese animals that performed exercises had significant improvement (p<0,05) in BMC of femurs, while non-obese animals that performed exercises showed improvement in BMC of femurs and tibias, aBMD of femurs and tibias, and the maximum allowed force of tibias. The suspended animals had a significant bone quality loss (p<0.05) in all parameters analysed. We can conclude that lack of use of a bone segment in Wistar rats in old age, both obese and non-obese, leads to a rapid weakening of all bone parameters. Exercise provides moderate positive effect in non-obese animals and low positive effect in obese animals.

Disclosure: The authors declared no competing interests.

P26

Restoring Bone Tissue Quality: a Treatment Target in Long-Term Bisphosphonate Therapy?

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Reaching a given BMD level is considered a treatment target. Bone quality is the other key component of bone strength. In patients on long-term oral bisphosphonates (LTB) we explore if bone quality, measured by microindentation, is associated with absence of fracture while on treatment (FWOT) and can be a treatment target. Cross-sectional study of osteoporosis patients on active LTB (>4 years) with good adherence and healthy volunteers. Other bone drugs or secondary

osteoporosis cases were excluded. Local ERB approved the study. Microindentation with an Osteoprobe® (Active Life Scientific, Santa Barbara, CA) was performed in the anterior midtibia after local anaesthesia, measuring Bone Material Strength index (BMSi) as previously described (Bridges 2012). Multivariate logistic regression was used to study the association between BMSi and FWOT amongst long-term OBP users. Area under ROC curve (AUCROC) was used to study discriminatory ability of BMSi and BMD for FWOT identification. Seventy-four subjects were included: 40 LTB (4 to 14 years exposure) users (18 no fracture WOT, 22 FWOT), and 34 controls. In univariate analysis, controls showed (mean, 95%CI) significantly higher BMSi (87.6 (85.1,90.2)) than LTB without FWOT (81.6 (78.5,84.8)), with fracture cases having significantly lowest BMSi (72.2 (67.8,76.5)). Similar trend (but no significant differences) were observed for spine and hip BMD. In multivariate models including only OBP users, higher BMSi was associated with reduced risk of FWOT, even after adjustment by age, years on BP, BMI and total hip BMD (adjusted OR 0.78/unit [0.64-0.94]; p=0.011). Conversely, BMD was not significantly associated with FWOT. BMSi had an AUCROC of 82.3%, compared with BMD (69.9% to 63.9% at different sites). In conclusion, better bone quality as measured by BMSi is inversely associated with FWOT amongst LTB users. Bone tissue quality levels, measurable by microindentation may be a treatment target although prospective validation studies are required.

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P27

Bone Material Strength as Measured by Microindentation *In Vivo* is Decreased in Patients with Fragility Fractures Independently of Bone Mineral Density

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Background: Bone Mineral Density (BMD) does not fully capture fracture risk, as the majority of fractures occur in patients with osteopenia. This suggests that altered bone material properties may contribute to fracture risk, independently of BMD. Recently, the microindentation *in vivo* technique has been made available to assess these properties. Our aim was to evaluate the relationship between Bone Material Strength (BMS), measured by microindentation *in vivo*, and fragility fractures in patients aged ≥40 years with low bone mass.
Methods: BMS was measured by the microindentation *in vivo* technique. The 10-year fracture probability was calculated using the FRAX algorithm.
Results: Ninety patients (53 female), mean age 61.0 years (range 40.4-85.5 years) were studied. 55 patients had osteopenia, 35 had osteoporosis and 63 had sustained one or more fragility fractures. There was a negative relationship between BMS and age (r = -0.539, p<0.001) and with the FRAX 10-year fracture probability with and without inclusion of femoral neck BMD (r = -0.383, p<0.001 and r = -0.426, p<0.001, respectively). In the whole group, BMS was lower in patients with a fragility fracture than in non-fracture patients (79.9±0.6 vs. 82.4±1.0, p=0.032) despite similar BMD. This was also the case in patients with osteopenia (80.3±0.7 vs. 83.9±1.2; p=0.015). There was no significant difference in BMS values in patients with a fragility fracture whether they had osteopenia or osteoporosis (79.8±0.8 vs. 78.7±1.1, p=0.456).
Conclusion: Our data suggest that patients with fragility fractures have altered material properties, as reflected by lower BMS, which are not captured by BMD. Further studies are required to establish whether BMS is of value in predicting fracture risk, particularly in patients with osteopenia.
Disclosure: The authors declared no competing interests.

P28

Prediction of Vertebral Body Strength in Patients with Multiple Myeloma using Finite Element Models with Specialized Cortical Element

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Multiple myeloma (MM) is associated with vertebral fracture. Finite element (FE) modelling simulates mechanical loading to directly estimate strength; however, the thin cortical wall, with a thickness on the order of the pixel size of clinical CT scanners, cannot be fully resolved. We developed an FE model with thin cortical elements and applied it to a dataset with MM patients. Vertebral quantitative computed tomography (qCT) scans were evaluated in 101 MM patients. An FE mesher was developed (Matlab) to generate nonlinear 'specialized-cortex' (SC) FE models from the images. The spongiosa region was meshed with tetrahedral elements, while the cortical mesh was created by applying pentahedral elements (triangular prisms) to the spongiosa surface. The element-level elastic modulus, failure stress and post-yield behavior were set from the local BMD according to relations from Keyak et. al., 1998. The patient-specific thickness of the cortical elements was determined from the density-weighted thickness and the elastic modulus set as 10GPa. A tetrahedral 'full-vertebral' (FV) mesh was generated with no special consideration for the cortex for comparison. Uniaxial compression was simulated (Calculix v.2.7) and the apparent level stiffness, yield load and work to yield as well as stress distribution were determined. Comparisons of the two models were made using linear regression and t-tests. Strong correlations were observed between the two models for stiffness (R^2=0.66), and yield force (R^2=0.61), with more moderate correlations observed in work to yield (R^2=0.39). The FV mesh showed significantly higher extrinsic stiffness (p=0.006) and yield force (p=0.02), while the SC model predicted a higher work to yield (p=0.002). In the SC model, the mean von Mises (VM) stress within the spongiosa and cortex were both associated with yield load (R^2 = 0.51 and 0.36, respectively). The distribution of the VM stress in the spongiosa (R^2=0.17) but not in the cortex (R^2=0.007) was associated with yield load. All parameters correlated significantly with BMD, with the correlation coefficents consistently higher in the FV model. Similar predictions of mechanics were obtained with both models;

however stiffness and strength may be overestimated and energy absorption underestimated when the cortical geometry is not taken into account. In MM patients, the strength is affected by the stress distribution in the spongiosa but not in the cortex, indicating that stress raisers in the spongiosa may be the leading cause of fracture in these patients.
Disclosure: The authors declared no competing interests.

P29
Bone Material Properties are an Independent Determinant of Fracture Risk and Vertebral Fracture Severity in Osteoporosis
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Bone mass, bone architecture and bone material properties are the principal determinants of bone strength. In this study we tester whether bone material properties can be considered an independent risk factor of osteoporotic fracture and also whether it predicts fracture severity. We performed microindentation at the anterior tibia, using (Osteo Probe III) in 41 controls and 61 patients with postmenopausal osteoporosis, (20 cases with hip fracture, 30 cases with vertebral fracture and 11 patients with non-vertebral non-hip fracture). The indentation distance increase was standardised into a calibration phantom (poly methyl-methacrylate) and converted to (Bone Material Strength (BMS) units). Bone mineral density (BMD) was measured by dual X-ray absorptiometry. Vertebral fracture severity was determined by semi-quantitative (SQ) grading of compression fractures on lateral X-rays of the DXA-scanner. BMS was found to be significant lower in subjects with osteoporotic fractures than in controls (77 ± 7.1vs 71.2 ± 6.5 $p < 0.05$). A significant negative correlation was observed for BMS on fracture SQ severity ($r^2 = 0.234$, p=0.012). After adjusting for age and lumbar spine BMD, ($r^2 = 0.153$, $p = 0.012$). For BMS, each incremental decrease of one unit in BMS was associated with a 9% increase in the likelihood of exhibiting fracture (OR 0.91, 95% CI 0.85, 0.97 $p = 0.005$). In the fracture group, BMD was lower at the total hip (0.784 ± 0.10 vs 1.161 ± 0.10 in controls; $p<0.05$) and spine (0.855 ± 0.194± vs 1.185 ± 0.178; p = 0,025). Serum markers of bone turnover (CTX and P1NP) were elevated in the fracture group vs. controls ($p = 0.035$ and p=0,016), respectively. Regression analysis revealed that BMS-values did no vary with BMD, age, or markers of bone turnover. In conclusion, bone material properties constitute an independent risk factor for osteoporotic fractures and severity of vertebral fractures.
Disclosure: The authors declared no competing interests.

P30
Vertical Ground Reaction Force During Standing and Walking: are they related to Bone Mineral Density Left Right Asymmetries
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Background: It is commonly assumed that there is minimal variation between both hips' bone mineral density (BMD). Until recently, screening was only done on one side, but due to technological development the study is now concurrently done on both hips. Dual-energy X-ray absorptiometry (DEXA) scan is routinely used to evaluate Bone Mineral Density (BMD) for the clinical diagnosis of osteoporosis. During standard BMD screening, we have noticed significant differences between hips in about 10% of the cases. The purpose of the current study was to determine whether asymmetrical femoral neck BMDs are also associated with asymmetrical gait.
Method: Study population included subjects with a difference higher than 0.5 SD in BMD between hips and normal control (less than 0.3 SD). All the participants performed gait tests. Exclusion criteria included any known neurological disease, leg operation, periheral neuropathy and orthopaedical problems. During the gait test subjects were asked to walk comfortably while outfitted with Pedar® pressure sensitive insole system (novel GmbH(. The subjects were also asked to walk at different speeds and while performing simple tasks. A number of gait parameters (for example foot pressure, step length) were recorded. For each gait parameter, we calculated an asymmetry index: GA (Gait Asymmetry) =| ln(XL/XR)|*100%. Values of 0.0 reflect perfect symmetry and higher values reflect greater degrees of asymmetry.
Results: The asymmetrical BMD group consisted of 36 participants (9 males; age 62.2 ± 9.89 years; BMI: 25.86 ± 5.28; Z scores between hips: 1.08±0.52). The symmetrical BMD group consisted of 9 participants (2 males; age 59.58 ± 5.1 years; BMI: 25.54 ± 4.31; Z scores between hips: 0.17±0.12). Most of the subjects were not aware of any difference between hips that could otherwise readily explain any gait asymmetries. The asymmetry indices of mean force, max force, step duration and swing time were significantly higher in the Asymm BMD group, compared with the Symm BMD group ($p<0.01$). Swing time difference (RSWT-LSWT) between left and right feet were correlated with BMD delta Z scores (Spearman's correlation = 0.38, $p = 0.02$.(There was no association between the side with lower BMD score and lower weight bearing (based on Ground reaction forces scores) according to preliminary results.
Conclusions: These preliminary findings may imply that asymmetries in BMD of the hips are associated with asymmetries in gait parameters and in the vertical ground reaction forces. These differences are naturally not the effect of nutrition, metabolism or lifestyle, which similarly affect both hips (significant left right differences were observed in particular

group of our patients). Future work should aim to identify the mechanisms underlying the relationship between these asymmetries (e.g., does one cause the other?), if gait and balance testing can aid in the early detection of compromised bone health, and whether appropriate gait training and exercises that alleviate gait asymmetries may reduce BMD asymmetries.
Disclosure: The authors declared no competing interests.

P31
Glucose-Dependent Insulinotropic Polypeptide (GIP) is Required for an Optimal Bone Quality
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Background: Rapidly after meal ingestion, gut hormones are release in the blood stream and induce a response in target cells expressing the gut hormone receptor. Among them, a role for the glucose-dependent insulinotropic polypeptide receptor (GIPr) in controlling bone strength and quality has previously been reported. GIPr KO animals presented with higher trabecular bone mass and reduction in cortical thickness. Nevertheless the tissue mineral density and the extent of collagen cross-linking were dramatically altered in trabecular and cortical bone. However, a second animal model of GIPr deletion exists and the opposite phenotype has been reported in this experimental model. The aims of the present study were to decipher the role of GIP in bone physiology by using an animal model of GIP deletion (GIP-GFP KI) or animals deprived in GIP-producing cells (GIP-DT).
Methods: Eight GIP-GFP KI and GIP-DT mice (16-week old) were age- and sex-matched with eight wild-type (WT) littermates. Trabecular and cortical bone microarchitecture were studied by high resolution microCT at the femurs and tibias. Bone strength was investigated by three-point bending. Non-parametric Mann-Whitney U-test was used to compare differences between groups.
Results: As compared with control mice, GIP-GFP KI animals exhibited significant reductions in BV/TV (22%, $p<0.001$), trabecular number (19%, $p<0.001$) and higher value for trabecular separation (14%, $p=0.007$). Surprisingly, these modifications of trabecular microarchitecture were not seen in GIP-DT animals, lacking GIP but also any other gut hormones made by K cells. Investigation of cortical microarchitecture revealed a significant reduction in cortical thickness in GIP-GFP KI mice (16%, $p=0.005$) whilst the same parameter was reduced in GIP-DT animals without reaching statistical significance. Furthermore, bone strength assessed by 3-point bending highlighted reduction in stiffness almost significant in GIP-GFP KI mice (10%, $p=0.07$) but not in GIP-DT animals ($p=0.28$).
Conclusions: These results support a role for the GIP/GIPr pathway in controlling bone strength and quality. However, these data also suggest that GIP-DT animal have a compensatory mechanism to maintain bone quality in the absence of GIP.
Disclosure: The authors declared no competing interests.

P32
Well-Controlled Premenopausal Type 1 Diabetic Women have no Deterioration of Bone Quality Measured by *In Vivo* Microindentation
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Type 1 diabetes mellitus (T1DM) is associated with increased risk of hip fracture. We aim to analyse the material properties by microindentation in women with T1DM and healthy controls as well as their correlation with BMD. Cross-sectional study including 19 well-controlled (by HbA1c) T1DM premenopausal women and 21 healthy women controls matched for age and Body Mass Index (BMI). Bone microindentation (Osteoprobe, Active Life, Santa Barbara, CA, US), bone mineral density (DXA) and levels of 25 OH vitamin D, calcium, phosphate and iPTH were measured. In the T1DM group, glycated haemoglobin, number of hypoglycaemias, chronic complications, and presence of metabolic syndrome (MetS) according to NCEP-ATPIII criteria were analysed. Bone microindentation on the midtibia, after local anaesthesia, measured Bone Material Strength index (BMSi) as previously described (Bridges 2012). Local ERB approved the protocol and informed consent was obtained. Age (34.5 ± 6.5 vs. 38.4 ± 9.9), BMI, 25 OHD, iPTH, calcium and phosphate levels were similar between groups. No significant differences (mean $\pm$ SD) were observed between T1DM patients and controls in BMSi (79.2 ± 11.2 vs. 80.3 ± 7.1) or BMD at lumbar spine (1.032 ± 0.078 vs. 1.008 ± 0.128) total femur (0.918 ± 0.098 vs. 0.857 ± 0.110) or femoral neck (0.817 ± 0.098 vs. 0.789 ± 0.129). In 5 patients with T1DM and microangiopathy compared with patients without, a trend towards lower BMS was observed (73.4 ± 13.0 Vs. 80.9 ± 10.1), although this difference was not statistically significant. Moreover, a tendency to lower BMS with increasing number of MetS criteria was seen. In conclusion, premenopausal women with well-controlled T1DM have bone tissue properties comparable to controls. There is a trend towards poorer bone mechanical properties in patients with T1DM and microangiopathy or MetS, suggest that worse control of disease may lead to worse bone quality.
Disclosure: ADP is advisor of Active Life Scientific. Supported in part by RETICEF, Instituto Carlos III, Spanish Ministry of Economy and Competitivity.

P33

Wolff's Law and the Interplay between Bone Structure and External Loading

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Although the proposition of Wolff's law as bone's ability to adapt its structure to mechanical needs is nowadays hardly questioned, the consequences of adaption in mechanical terms resist a clear-cut description. To understand the interplay between the habitual loading conditions on the bone and the local bone structure, the proximal femora of four primates with dissimilar locomotor habits were imaged with high resolution micro-computed tomography. A previous study revealed different strategies of how the bone volume fraction (BV/TV) of the trabecular bone is locally adapted: high values of BV/TV are obtained by a thickening of the trabeculae with trabecular number (Tb.N) being relatively constant, while low BV/TV values are obtained by a reduction of Tb.N, whereas Tb.Th remains constant.[1] Here we go beyond a structural analysis and calculate the local tissue strains using microfinite element analysis. Proper boundary conditions reflecting prevalent loadings were estimated based on muscle insertion locations and pulling directions. The forces were applied on the femoral head and great trochanter with magnitudes proportional to the animal weight. The design of the computer experiment was to test the mechanical properties of all 16 combinations of the four proximal femora under the four different loading conditions. In all animals, the femoral neck always showed not only the highest strains but also the most heterogeneous strain distributions. The strain distributions did not provide features that clearly indicated when there was a match between bone structure and loading conditions. A comparison between the different scenarios further allows a consideration whether differences in bone structure or differences in the external loading has a stronger influence on the resulting strains.

Disclosure: The authors declared no competing interests.

Reference

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P34

Changes in Fase Content of the Biominerals in Rat Pelvic Bone after 60-Days' Influence of the Epychlorhidrine Vapours and Possible Ways of its Correction

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Background: The aim was to study the reaction of the biomineral fractions in the pelvic bones of the rats of the different ages under the 60-days influence of the epychlorhidrine (E) vapours and possible correction by thiotriazoline (Th) and Echinacea Purpura (Ech) tincture.

Methods: Four hundred and twenty white male rats were divided into three groups including ages: young, reproductive, old. 1 group stay intact; in 2ndgroup– rats have the inhalations of the E at the dose 10 DL, 5 hours/daily; 3rd–4thgroups – rats were injected 2.5% Th at the daily dose 117.4 mg/kg or the Ech at the daily dose 0.1 mg per 100 g of the body weight orally. Experiment finished at the 1, 7, 15, 30 and 60 days via the decapitation of the rats. Pelvic bones were fractionised and revised by X-ray structure analysis. The content of the hydroxylapathite, vithlokite and calcite were measured. Data were processed by statistical analysis.

Results: After the E inhalations in young rats, the vithlokite and calcite content in bone mineral matrix were over the control rates by 9.15% - 15.60%, in old rats – by 6.48% and 8.64%. The hydroxylapathite content exceed the control rates by the 4.95%, 5.20% and 3.12%. After the finish of the inhalations during the re-adaptation period in young and reproductive animals rats the biomineral status was improved to the control levels, and in old rats the biomineral content remained unimproved, but administration of Th or Ech obviously have modulated the biomineral status towards the reparation. Application of the Th during the E inhalations increased the crystallisation in the bone minerals through the whole re-adaptation period, administration of the Ech increase the mineralisation only temporary.

Conclusion: Administration of Th or Ech prevents the distortion of the bony biominerals after the 60-days' influence of the epychlorhidrine vapours, but Th has more reparative potency.

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P35

Bone Quality is Altered by Hypoactivity in the Chicken: a FTIR study

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Background: Disuse induces a rapid bone loss in adults; sedentarity is now recognised as a risk factor for osteoporosis. Hypoactivity or confinement also decrease bone mass in adults but their effects are largely unknown and only few animal models have been described. The hypodynamic chick confined in small cages has been recognised as a suitable model of bone loss during growth. However, the effects on the quality of the bone matrix have not been studied.

Methods: We have used 10 chickens of the rapidly growing strain 857K bred in a large enclosure (FREE group); 10 others were confined in small cages with little space to move around (HYPO group). They were sacrificed at 53 days and femurs and tibias were evaluated by microcomputed tomography (microCT) and histomorphometry on undecalcified bone sections. Sections (4 µm thick) were analysed by FTIR to see the effects on mineralisation and collagen.

Results: Hypoactivity had no effect on the length and diameter of the bones. Bone mass measured by microCT (trabecular bone volume and trabecular microarchitecture) was significantly reduced in the animals of the HYPO group. An increase in osteoid volume and surfaces was noted in

the HYPO group. However, there was no alteration of the calcified volume as the osteoid thickness did not differ from control animals. FTIR showed a significant reduction of the mineral to matrix ratio (band 900-1200cm^{-1} / Amides I band at 1590-1720cm^{-1}) in the HYPO group associated with an increase in the carbonate content and an increase in crystallinity indicating a poor quality of the mineral when compared with the FREE group. Collagen maturity (calculated from the ratio of intensity sub-bands ratio at 1660 and 1690cm^{-1}) was significantly reduced in the HYPO group.
Conclusions: The confined chicken represents a new method for the study of hypodynamia since disuse is not created by a surgical lesion nor a traumatic method such as bandaging or special cages. Animals have a reduced bone mass but also present an altered quality of the bone matrix which appears less mineralised and whose collagen contains less cross-links than control chicken as evidenced by FTIR.
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P36
Bone Loss Comparing Two Experimental Protocols that Induce Osteopenia
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Many studies use different experimental protocols to induce osteopenia in rats, among which the most used are: tail suspension and ovariectomy. Our goal was to determine whether the different causes of osteopenia have the same effects on bone structure. For this study, 30 females Wistar rats of 19 weeks of age, were distributed into the groups: control group (GC), group suspension (GS) and ovariectomised group (OVX). The tail suspension was performed for 21 days to 24 weeks of age and the euthanasia of all animals was performed at 27 weeks of age. Bone mineral density (BMD) and biochemical markers of calcium, phosphorus, and alkaline phosphatase were analysed at the beginning and at the end of the experiment. The result showed that S femoral BMD decreased compared with the C ($p = 0.0350$), while that at OVX decreased compared to the beginning of the experiment ($p = 0.0114$), however was no difference with the control group ($p = 0.3940$). By analysing the tibia, BMD in GS and OVX compared with the CG did not reach statistical difference, however BMD in SG decreased compared with the beginning of the experiment ($p = 0.0469$). Biochemical assays showed that the plasma concentration of calcium was lower after the experiments in OVX and SG groups ($p = 0.0318$ and $p = 0.0320$, respectively), but both had no difference between the control. The phosphorus concentration of SG animals had a significant increase ($p = 0.0246$), while the other groups maintained their concentration equilibrium. Finally, the activity of alkaline phosphatase in OVX rats was significantly higher compared to the beginning of the experiment ($p = 0.0427$) whereas in SG there was a significant decrease ($p = 0.0078$). We can conclude that in female rats, the severity of bone loss was greater in the tail suspension model.
Disclosure: The authors declared no competing interests.

P37
Greater Anthropometric Parameters of the Pelvis and Limbs are Associated with Higher Bone Mineralisation in Young Indians
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Background: To compare the relationship of the somatometric parameters of the appendicular and axial skeleton with the bone mineral status in ethnically equal Indian group of 17 to 21 year old boys.
Methods: Two hundred and forty one healthy boys had assessments body mass, height, transverse, longitudinal and circumference (Cir) body measurements. Whole body bone mineral density (BMD, g/cm^3) and content (BMC, g) were assessed using dual-energy X-ray absorbtiometry (DXA). Pearson's correlation coefficient (CC $r_{x/y}$) was estimated between mentioned above anthropometric data assessed by the Statistic program software Excell-2007.
Results: In modern young Indian males the BMD reaches 0.94±0.02, the BMC 67.03±1.96, the mean body weight was 64.90±0.74 kg and highly correlated with the body Cir such as chest, gluteal, forearm and thigh Cir (CC $r_{x/y}$ 0.66). Greater body mass was not associated with greater bone mineral content (CC $r_{x/y}$ 0.75, $p > 0.05$). The body height in Indian boys takes 171.33±0.78 cm and has no evident correlations with the rest neither somatometric measurements and BMD or BMC, so stated as the independent body parameter. The upper limb's length directly proportional to the lower limb's length; the length of the tight has the direct proportionality to the pelvic size (CC $r_{x/y}$0.58-0.54) and strong correlation with the BMD (CC $r_{x/y}$ 0.50, $p<0.05$) and BMC (CC $r_{x/y}$ 0.47, $p<0.001$). The length of the pelvis also strongly correlates with the BMD (CC $r_{x/y}$ 0.46, $p<0.05$).
Conclusion: The BMD and BMC significantly associated with the pelvic size, length of the lower limb, but stay independent from the total body parameters as the body weight or height. The BMD and BMC are mostly predicted by the anthropometric parameters of the tubular bones of the limbs.
Disclosure: The authors declared no competing interests.

P38
Identification of Pyridinoline Trivalent Collagen Cross-Links by Raman Microspectroscopy
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The intermolecular cross-linking of bone collagen is intimately related to the way collagen molecules are arranged in a fibril, determines certain mechanical properties, and has been suggested to be involved in the mineralisation initiation process. Once type I collagen has been synthesised by osteoblasts, it undergoes extensive posttranslational modifications,

resulting in a characteristic pattern of cross-links. The post-translational modifications of the organic matrix are important for both its structural and mechanical properties, and disruption of the cross-linking can result in dysfunction of the tissue. Raman microspectroscopy allows the analysis of minimally processed bone blocks and provides simultaneous information on both the mineral and organic matrix (mainly type I collagen) components with a spatial resolution of ~ 1 µm. The purpose of the present study was to validate Raman spectroscopic parameters describing one of the major mineralising type I trivalent cross-links, namely pyridinoline (PYD). To achieve this, a series of collagen cross-linked peptides with known PYD content (as determined by HPLC analysis), porcine skin, and porcine predentin and dentin were analysed by Raman microspectroscopy. Spectra were further processed by means of difference and second derivative spectral resolution methods. The results of the present study confirm that it is feasible to monitor PYD trivalent collagen cross-links by Raman spectroscopic analysis in mineralized tissues, through a spectral components ~ 1660 wavenumbers exclusively. This allows the determination of the relative pyridinoline content in undecalcified bone tissues with a spatial resolution of ~ 1 µm, thus enabling the correlation with histologic and histomorphometric parameters.
Disclosure: The authors declared no competing interests.

P39

Effect of One Year Cross-Sex Hormonal Treatment on Bone Mineral Density of the Lumbar Spine in Transgender Patients

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Background: Oestrogen can increase bone mineral density (BMD) by decreasing bone turnover, which is mainly seen in trabecular bone. Testosterone can increase bone size, but the effect on BMD is less clear. Cross-sex hormonal treatment (CSHT) in transsexuals can therefore affect the BMD. For example, in male-to-female individuals (MtFs) a lower BMD before start of CSHT has been described in comparison to healthy control men. The objectives were to investigate the effects of CSHT on BMD during the first year of hormonal treatment in MtFs and female-to-male individuals (FtMs).
Methods: Prospective study, part of ENIGI (European Network for Investigation of Gender Incongruence). 74 adult patients who completed one year of CSHT were included. In 37 FtMs and 37 MtFs a dual-energy X-ray absorptiometry (DEXA) was performed to measure the BMD of the spine at start and after a year of CSHT. The FtMs received testosterone undecanoate intramuscular (i.m.) (1000 mg/12 weeks), testosterone gel (50 mg/day) or testosterone esters i.m. (250 mg/2 weeks). The MtF group was treated with oestradiol valerate (2-4mg/day) or an oestradiol patch (200ug/week) and most MtFs received cyproteronacetate (50mg/day) simultaneously.
Results: At baseline the BMD of the spine of the FtMs was 0.99 g/cm^2, (SD±0.10) and after one year 1.00 g/cm^2, (SD±0.10) reflecting a mean difference of 1 % (95% CI -0.31 to 2.24). In the MtFs at baseline and after one year, the BMD of the spine was 1.00 g/cm^2, (SD±0.11) and 1.03 g/cm^2, (SD±0.11) respectively, reflecting a mean difference of 3.6 % (95% CI 2.30 to 4.86).
Conclusion: In FtMs the BMD of the spine remained stable after one year CSHT. In the MtFs group, the BMD increased on average with 3%. Taken into account the short study period, the change in BMD suggests that BMD is an important variable in the follow up of transsexuals.
Disclosure: The authors declared no competing interests.

P40

Posterior Mandibular Alveolar Ridge Augmentation: an Onlay Technique Using Autogenesis Intra Oral Mandibular Tori (Benign Bone Exostosis)

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Background: When considering implant rehabilitation for missing teeth both bone volume and quantity needs to be considered for placement. With natural atrophy of the alveolar ridge at sites of missing teeth bone augmentation can be required prior to implant placement. The use of iliac crest, mandibular ramus and chin as donor sites are well documented in the literature but are not without potential morbidities. We present a case report where mandibular tori (benign bone exostosis) are used as an onlay autograft for localised alveolar ridge augmentation in the posterior maxilla.
Methods: Thirty-nine-year old male presenting with hypodontia, mandibular tori and an atrophic region in the maxilla and mandible in the premolar region. The multidisciplinary team consisting of Orthodontics, Restorative and Oral and Maxillofacial surgery (OMFS) carried out full management.
Results: Orthodontics for initial deciduous extractions, alignment of the arches and optimisation of the recipient sites with removable retainers prior to operation. OMFS provided a single procedure of bilateral mandibular tori harvesting, onlay graft with titanium screw fixation along with bone scrapings and a particulate graft cover Bio Oss® prior to a resorbable non cross-linked membrane Bio Gide®. Restoratively a 2 stage implant placement with final restorative construct.
Conclusions: Although extra oral sites can be used they produce a second donor site which can give rise to complications. Common intra-oral sites include the maxillary tuberosity mandibular symphysis, and ascending ramus of the mandible which have been shown to have low associated morbidity. From the literature success of intraoral endosseous implant placement into autogenous bone grafted from intra-oral donor sites has been shown to be up to 98.3%. With the occurrence of mandibular tori reported between 6-32% this case has shown that they are a viable source for autogenous bone in alveolar bone augmentation.
Disclosure: The authors declared no competing interests.

P41

The Effects of Sodium Glutamate and Ionising Radiation on Strength of the Mandible in Rats

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Background: The aim was to investigate growth rates of bones in rats in readaptation period after application of sodium glutamate (SG) and exposure to ionising radiation (IR), and finding possibility of medication with Spirulina (Sp).
Methods: The experiment involved 240 rats with body weight of 180-200 g. The animals were distributed into 8 groups as follows: intact animals for the controls, animals that received *per os* SG in dosage of 30 mg per kg daily for 60 days, animals exposed to IR (total 4 Grey in 4 sessions), received Sp in dosage of 250 mg per kg, combined SG and IR, SG and Sp, Sp and IR, and all three agents simultaneously. The animals were withdrawn from the experiment by the 1st, the 7th, the 15th, the 30th, and the 60th day after cessation of experimental influences. The mandibles were excised and put to strength testing at bending (V.G. Koveshnikov, V.I. Luzin, 2003).
Results: Upon SG discontinue, ultimate bending strength, elasticity modulus, and fracture energy were lower than those of controls by 7.29%, 6.24% and 6.32%; after IR discontinue same values were lower by 8.32%, 9.75% and 7.86%. After combined action of SG and IR those values were lower by 12.15%, 11.94% and 12.22% as compared with controls. Restoration of strength features also depended on influence: by the 60th day after SG discontinue significant differences from the control values were not observed, after IR discontinue some differences were still observed, and after cessation of combined action strength features did not recover. Application of Sp reduced negative effects of experimental conditions on strength of mandible. The best recovery outcome was observed in animals that received only SG and the lowest recovery outcome was yielded in rats exposed to combined action of IR radiation and SG.
Conclusions: 60-day application of SG in dosage of 30 mg per kg of body weight and exposure to IR and their combined action results in marked decrease of bone strength that expands even to readaptation period. This fact urges searching for medication and prophylactic measures for such a state. According to our findings Sp well satisfies this demand.
Disclosure: The authors declared no competing interests.

P42

Differences in Volumetric Bone Mineral Density (vBMD) at the Radius and Tibia in Premenopausal Caucasian, South Asian and Arab women

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Current studies on ethnicity bone health indicate Middle-Eastern females are at high risk of low bone density among premenopausal groups.[1] However, limited data is available regarding the young female groups with comparison to other ethnic populations. This study is a follow up measurement where pQCT scans of Arab females have been measured and compared with older pQCT scans to investigate the differences between volumetric bone mineral density (vBMD) between Caucasian (C), South Asian (SA), and Arab (A) women. Fifty-seven healthy premenopausal women (22 C, 19 SA

Table1 [P42]:

		Caucasian (n=22)	South Asians (n=19)	Arab (n=16)
pQCT 4% radius	TOT_DEN (mg/cm3)	315.6(45.3)	312.1(45.6)	303.1(50.3)
	TRAB_DEN (mg/cm3)	178.9(35.5)	175.3(37.2)	171.6(31.6)
pQCT 66% radius	TOT_DEN (mg/cm3)	746.6(86.3)	768.8(85.9)	709.7(65.4)
	CORT_DEN (mg/cm3)	1134.7(38.4)	1126.3(42.5)	1111.6(47.0)
pQCT 4% tibia	TOT_DEN (mg/cm3)	303.3(31.7)[b]	343.6(53.4)[a]	278.6(32.4)[b]
	TRAB_DEN (mg/cm3)	228.7(29.2)	272.4(66.9)	205.1(37.2)
pQCT 14% tibia	TOT_DEN (mg/cm3)	571.6(117.4)	495.5(92.1)	552.8(131.2)
	CORT_DEN (mg/cm3)	1135.6(24.4)[b]	1095.0(65.5)[a]	1143.1(19.4)[b]
pQCT 38% tibia	TOT_DEN (mg/cm3)	903.6(76.9)	856.8(85.2)	862.5(65.9)
	CORT_DEN (mg/cm3)	1179.8(19.4)	1160.9(53.3)	1187.3(26.9)

and 16 A), age range 18-55 yrs, were studied. Peripheral Quantitative Computed Tomography (pQCT) measurements were taken at the radius and tibia (non-dominant) using a Stratec XCT 2000 pQCT scanner. The Table 1 below shows the results of vBMD at 4% and 66% radius; 4%, 14%, and 38% tibia in C, SA and A women.

Cells within a row with different superscript letters are significantly different. Data expressed as the mean (SD); Abbreviations: TOT_DEN, total density; TRAB_DEN, trabecular density; CORT_DEN, cortical density. SA had significantly higher total density at 4% tibia than C and A women but significantly lower cortical density at 14% tibia site than C and A women. There were no significance differences in vBMD observed between the ethnic groups in radius sites (not shown in the table). However, at 4% radius, Caucasian women had significant higher bone mass ($p<0.01$) and total area ($p<0.05$) and trabecular area ($p<0.05$) than SA and A women. Our novel findings for differences in the radius and tibia sites C, SA, and A premenopausal women require further investigations, particularly with respect to future fracture risk.

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P43

Morphological, Densitometric and Mechanical Properties of Femur, Tibia and Tarsometatarsus in Female Ostriches (*Struthio Camelus*)

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The aim of this study was comparison of morphological, densitometric and mechanical properties of femur, tibia and tarsometatarsus in 14-month-old female ostriches (N=13). Bone length, bone weight and relative bone weight (RBW) were determined. Using quantitative computed tomography technique, cortical bone mineral density (Cd), mean volumetric bone mineral density (MvBMD), total bone volume (Bvol) and calcium hydroxyapatite density (Ca-HA) in the cortical bone were measured. Bone mineral density (BMD) and bone mineral content (BMC) were evaluated using dual-energy X-ray absorptiometry. Cortical bone area (CBA), cross-sectional area (A), second moment of inertia (Ix), mean relative wall thickness (MRWT) and cortical index (CI) were derived from the measurements of horizontal and vertical diameters of the investigated bones in the midshaft. Using three-point bending test, mechanical parameters of the bones (maximum elastic strength (Wy) and ultimate strength (Wf)) were determined. Bone weight, RBW, Bvol, BMC and Ca-HA of tibia were significantly higher when compared with these parameters in femur and tarsometatarsus ($P\leq0.01$). Bone length and BMD reached the highest values in tibia than in tarsometatarsus and femur (both $P\leq0.01$). MvBMD was the highest in tarsometatarsus than in tibia and femur (all $P<0.001$). Ix reached the highest value in femur than in tibia and tarsometatarsus (all $P\leq0.01$). Cd, CBA, A and Wy were significantly lower in tarsometatarsus than in femur and tibia ($P<0.05$). MRWT and CI were significantly lower in femur when compared with tibia and tarsometatarsus ($P<0.001$). Wf of tarsometatarsus was significantly lower than in tibia ($P=0.01$). The obtained results have shown significant differences between morphological, densitometric and mechanical properties of the evaluated bones in female ostriches. Determination of morphological, densitometric and mechanical properties of femur, tibia and tarsometatarsus in female ostriches may serve for further studies on metabolic regulation of skeletal system properties with environmental, physiological, dietary, pharmacological and toxicological factors.

Disclosure: The authors declared no competing interests.

P44

Sex-Related Differences of Morphological and Densitometric Properties of Mandible in Silver Foxes (*Vulpes Vulpes*)

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Considering limited information available on skeletal system properties in silver foxes, the aim of this study was to determine morphological and densitometric parameters of mandible obtained from males and females. The study was performed on 1-year old male (n = 7) and female (n = 8) silver foxes. Mandible was isolated and its weight and length were determined. Bone mineral density (BMD) and bone mineral content (BMC) for whole mandible, mandible body and mandible ramus were determined using dual-energy X-ray absorptiometry (DEXA) method and Norland XR-46 Densitometer (Fort Atkinson, WI, USA) equipped with Research Scan

software. Statistical comparison of the investigated parameters of mandible between males and females was performed with a use of non-paired Student t-test and $P < 0.05$ was considered as statistically significant. Final body weight was significantly higher by 20% in males than in females ($P = 0.004$). Mandible length and weight were significantly higher in males by 7% and 22%, when compared to the group of females, respectively ($P < 0.001$). BMC measured for whole mandible and for its ramus reached significantly higher values in males by 10.5% and 18.3% when compared with the females ($P \leq 0.01$). In conclusion, this study has shown sex-related differences of body weight as well as length, weight and BMC values of mandible in silver foxes. This study provides data on basic anatomical and densitometric properties of mandible in male and female silver foxes. The obtained results indicate that silver fox may serve as an attractive experimental model for further studies on bone metabolism regulation in mammals in response to physiological, environmental, pharmacological, nutritional and toxicological factors, being an alternative model for other monogastric animal species such as dogs.

Disclosure: The authors declared no competing interests.

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Comparison of Morphological, Densitometric and Mechanical Properties of Femur, Tibia and Tarsometatarsus in Male Ostriches (*Struthio Camelus*)

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This study compared morphological, densitometric and mechanical properties of femur, tibia and tarsometatarsus in male ostriches (N=5) kept to slaughter age of 14 months of life. Bone length, bone weight and relative bone weight (RBW) were determined. Using quantitative computed tomography technique, cortical bone mineral density (Cd), mean volumetric bone mineral density (MvBMD), total bone volume (Bvol) and calcium hydroxyapatite density (Ca-HA) in the cortical bone were measured. Bone mineral density (BMD) and bone mineral content (BMC) were evaluated using dual-energy X-ray absorptiometry. Cortical bone area (CBA), cross-sectional area (A), second moment of inertia (Ix), mean relative wall thickness (MRWT) and cortical index (CI) were derived from the measurements of horizontal and vertical diameters of the investigated bones in the midshaft. Using three-point bending test, mechanical parameters of the bones (maximum elastic strength (Wy) and ultimate strength (Wf)) were determined. Bone weight, RBW, Bvol, BMD and BMC of tibia were significantly higher when compared with these parameters in femur and tarsometatarsus ($P<0.001$). Bone length reached the highest value in tibia than in tarsometatarsus and femur (all $P<0.001$). MvBMD was the highest in tarsometatarsus than in tibia and femur (all $P<0.001$). Ix reached the highest value in femur than in tibia and tarsometatarsus (all $P<0.05$). CBA and A were significantly lower in tarsometatarsus than in femur and tibia ($P<0.01$). MRWT and CI were significantly lower in femur than in tibia and tarsometatarsus ($P<0.001$). Wy and Wf of femur, tibia and tarsometatarsus were not significantly different ($P>0.05$). This study showed significant differences between morphological and densitometric parameters of the evaluated bones in male ostriches. Determination of morphological, densitometric and mechanical properties of femur, tibia and tarsometatarsus in male ostriches may serve as an attractive model for studies on metabolic regulation of skeletal system properties with environmental, physiological, dietary, pharmacological and toxicological factors.

Disclosure: The authors declared no competing interests.

P46

A Novel Approach of Bone Density Analysis based on Bone Remolding

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Supporting our organs and human features, bones gradually adapt to their optimal structure. As external loadings change the bone structure will opt for the best shape and structure to adapt to the new loadings; this is called bone functional adaptation. Bones are not necessarily always healthy and may have defects or fractures. The implantation of bone material will cause changes to the stress upon the bones, which will in turn cause changes to the internal structure optimisation. The simulation results were obtained with different implant materials. When the elastic modulus of implant material was 0.002Mpa, the osteogenesis rate approaches to zero, which is consistent with the clinical results. When the defect exceeds threshold, it is harder to fill by its adaptability. When E=30Mpa, the asteogenic is less than the stiffer material because the tress is less. In the same degradation period, the effect of materials with about 1000Mpa is better than others. When the value up to 3000Mpa, the osteogenesis rate is down because the material degradation is slower and there is no place for bone. Theory and simulation techniques were obtained for the abnormalities situations of bone. Through computer simulation, two basic problems of matching were solved: how to quantify the impact of implant materials and how to fine the suitable biodegradable materials.

Disclosure: The authors declared no competing interests.

P47

Van Buchem's Disease, a Case Report

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Van Buchem is a very rare disease. It is an uncommon autosomal recessive disorder, characterised by hyperostosis of the skull, mandible, clavicles, ribs, and diaphyseal cortices of the long bones. The establishment of accurate diagnosis of bone dysplasias when the abnormal findings are demonstrated only radiographically can be difficult. The dominant form of the disease most often symptomatically confined to the mandible, which help diagnosis. This leads to facial nerve pulsy, hearing loss, and optic atrophy. We report a 31 year-old Moroccan man showed marked radiographic changes throughout the skeleton and had a square pagetoid face.

Disclosure: The authors declared no competing interests.

BONE DEVELOPMENT/GROWTH AND FRACTURE REPAIR

P48 (OP2)

P49 (OP7)

P50

Bovine Lactoferrin Increases New Bone Formation in a Rat Critical-Sized Calvarial Defect Model

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Background: Lactoferrin is an 80-kDa iron-binding glycoprotein that is produced in milk and other exocrine glands. *In vitro*, lactoferrin functions as a potent bone anabolic factor by increasing the proliferation, differentiation and survival of osteoblasts and by inhibiting osteoclastogenesis. *In vivo*, the effect of local application of lactoferrin on bone regeneration has produced conflicting results and studies thus far have only employed non-critical-sized calvarial defect or local subcutaneous injection models. The objective of this study was to assess the ability of bovine lactoferrin to increase bone regeneration in a rat critical-sized calvarial defect model.

Method: Critical-sized defects (5mm) were created over the right parietal bone in 60 sexually mature male Sprague-Dawley rats. The rats were randomised into three groups: Group 1(control) – defects were left empty; Group 2 – defects were filled with a 100 µl collagen gel (3 mg/ml); and Group 3 – defects were filled with a 100 µl collagen gel containing 10 µg bovine lactoferrin. The rats were sacrificed at 4 or 12 weeks post-operatively and the calvaria were harvested for assessment of bone regeneration using µCT. This study was approved by the local Animal Ethics Committee.

Results: Collagen gels had completely degraded in all animals at the time of sacrifice. The percentage of new bone formation, at both 4 and 12 weeks was significantly greater in the group treated with lactoferrin compared with other groups. The percentage of new bone formation in groups 1, 2 and 3 was 42.7±4.2%, 35.9±5.9% and 63.2±2.3%, respectively, at 4 weeks (P=0.0008); and 41.1±5.2%, 45.8±4.8% and 74.6±4.3%, respectively, at 12 weeks (P<0.0001).

Conclusion: This study demonstrated that local application of lactoferrin significantly increased bone regeneration in a rat critical-sized calvarial defect model. The profound anabolic effect of lactoferrin on bone regeneration has therapeutic potential for treatment of bony defects and fracture non-union.

Disclosure: The authors declared no competing interests.

P51

Antagonising Midkine Enhances Osteoblast Differentiation and Fracture Healing in Mice

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One growth factor that potentially plays a role in fracture healing is midkine (Mdk). Previous findings suggest that Mdk is a negative regulator of osteoblast activity and bone formation, thereby raising the possibility that a specific Mdk-antagonist might improve fracture healing. The aim of the present study was to evaluate the effects of a monoclonal anti-Mdk antibody (Mdk-Ab) on bone repair *in vivo* and on osteoblasts *in vitro*. Forty-two C57BL/6J mice received a diaphyseal femur osteotomy stabilised with an external fixator. Half of the animals were injected with vehicle (PBS) or Mdk-Ab twice per week for three weeks. The mice were sacrificed at day 10, 21 or 28 and fracture healing was assessed by 3-point-bending test, micro-computed tomography, histomorphometric and immunohistochemical analysis. For *in vitro* experiments, preosteogenic cells were differentiated for 5 days and recombinant Mdk and the Mdk-Ab were added for 6 h to the culture medium. Gene and protein expression was analysed using qPCR and western blotting. (n=6-8; p<0.05; Mann-Whitney-U test). During fracture healing, treatment with Mdk-Ab led to a significantly increased relative flexural rigidity after 21 and 28 days. Bone mineral density and bone volume ratio were significantly increased in the fracture callus at day 21. Histomorphometric analysis revealed that the bone content in the fracture callus was significantly increased after 10 and 21 days. Osteoblast surface as well as beta-catenin expression were also significantly increased. *In vitro* experiments confirmed a negative influence of Mdk on osteoblast differentiation and beta-catenin signalling that was abolished by Mdk-Ab treatment. Antagonising Mdk seemed to improve osteoblast function based on an increased beta-catenin signalling, leading to a faster callus mineralisation and therefore to an accelerated bone repair. The findings of the present

study indicate that there is a therapeutic potential for the Mdk-Ab to enhance fracture healing in patients with orthopaedic complications.
Disclosure: The authors declared no competing interests.
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P52
Two-Year Follow-Up of Fracture Healing in the Distal Radius in Post-Menopausal Women Using High Resolution Peripheral Computed Tomography
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High resolution peripheral computed tomography (HR-pQCT) in combination with finite element analysis (FEA) is a promising tool to assess the healing process of distal radius fractures. In a recently published pilot study, 18 fractures were scanned by HR-pQCT up to 12 weeks post-fracture. We here present the changes in bone parameters at the fracture side during a >2-year follow-up, compared with the same region at the contralateral radius. We included 18 post-menopausal women (mean age 64 years) with a stable distal radius fracture. HR-pQCT scans of the fracture region were performed at five visits scheduled at 1-2 (baseline), 3-4, 6-8, 12 weeks and >2 years post-fracture. At the last visit, the same region at the contralateral side was scanned as well. Bone density, micro-architectural and biomechanical parameters were calculated from the HR-pQCT scans in combination with FEA. Bone parameters at fracture side at each visit were compared to the bone parameters at contra-lateral side using a linear mixed-effect model. After >2-year follow-up the cortical fracture gaps were completely restored in all patients. The initial lower cortical density became comparable to the contra-lateral radius, while cortical thickness became higher (+20%, p=0.047). Trabecular density, number and thickness at fracture side were initially higher. While trabecular density and number decreased and became comparable with the contralateral radius, trabecular thickness remained higher at >2-year post-fracture (+52%, p<0.001). As a result, the initial lower torsional and bending stiffness became higher at the fracture side than at the contralateral radius after >2 years (+31%, p=0.016; and +29%, p=0.030, respectively). We conclude that during a period of >2 years post-fracture, fracture healing is still ongoing, with several cortical and trabecular bone parameters becoming higher than at the contralateral radius. This resulted in a restoration of bone stiffness at fracture side exceeding the stiffness at contralateral site.
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P53
Pro-inflammatory T Cells Stimulate Osteoblast Maturation *In Vitro*
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Background: The local immune response is an important pillar to consider when the aim is to enhance bone healing or regeneration. Although T lymphocytes are identified as positive regulators in fracture healing, it is not known whether they mediate their effects directly on osteoblast maturation. The goal of this study was to investigate the role of different T cell subsets on the osteogenesis of human mesenchymal stem cells (MSCs).
Methods: T cell populations were isolated from donor blood and activated. Naive T cells were polarised towards pro-inflammatory T helper 17 (T_H17) or anti-inflammatory regulatory T (T_{REG}) cells. The effect of T cell cytokines on osteogenesis of MSCs was studied in direct coculture or conditioned medium (CM) experiments. Alkaline phosphatase (ALP) activity and matrix mineralisation were measured as osteogenic markers. The role of cell-to-cell contact was studied in transwell culture assays. Moreover, cell proliferation was measured.
Results: T cells or their CM significantly increased the ALP activity in MSCs. In osteogenic medium, T cell CM enhanced their calcium deposition. CD4+ T helper cells had the largest stimulatory effect on the osteogenic differentiation of MSCs. Inhibition of cell-to-cell contact did not change the T cell-mediated effects. An enrichment in the number of T_{REG} cells had no additional effect on MSCs. In contrast, T_H17 polarisation increased the stimulatory effects of the T cell CM. Strong pro-osteogenic effects were observed when studying T_H17 cell-specific cytokines directly on MSCs.
Conclusion: The results of our experiments indicate that activated T cells are regulators of osteoblast maturation through the production of soluble factors. Moreover, individual T helper cell subsets differentially control MSC osteogenesis. We show that pro-inflammatory T lymphocyte populations, including the T_H17 cells, are highly stimulatory for osteogenic differentiation. The occurrence of different T cells may be a predictive factor for bone healing in patients.
Disclosure: The authors declared no competing interests.

P54

Nitric Oxide Synthase Deficiency Inhibits Callus Formation Resulting in Nonunion Development

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Background: In high-risk patients up to 45% of fractures heal inadequately, resulting in nonunion development with major consequences for patient's quality of life. To enhance bone healing, sufficient production of nitric oxide (NO), solely derived during the conversion of arginine into citrulline by nitric oxide synthases (NOSs), may be essential. NO stimulates bone cells to regulate bone remodelling, influences vascular reactivity and enhances collagen synthesis, all essential components of normal fracture healing.
Methods: In 20-24 week old wild type and either inducible or endothelial NOS (respectively NOS2 or NOS3) deficient mice (n=8/group), periosteal cauterisation and a 0.45 mm femur osteotomy was performed. Callus volume was measured using micro-computed tomography (μCT) after 7, 28 and 42 days of fracture healing. Myeloperoxidase was measured with immunohistochemistry to determine neutrophil influx in callus tissue and bone marrow. Femurs were also sampled for RNA analysis of relevant enzymes and high-performance liquid chromatography measurement of amino acids.
Results: After 28 and 42 days of fracture healing, NOS deficient animals showed evident nonunion, while union was reached in all wild type animals after 28 days. Both NOS knock out groups showed an increased neutrophil influx compared with wild type mice both at 1 week and 1 month of fracture healing. At these time points, amino acid concentrations of arginine, ornithine and citrulline, and expression of enzymes related to the arginine-NO metabolism were deregulated in NOS deficient animals when compared to wild type mice.
Conclusion: In the present study we showed for the first time that the absence of either NOS3 or NOS2 results in depleted substrate metabolism and an inadequate fracture healing. Enhanced myeloperoxidase levels indicate a role of disturbed inflammatory response in the development of nonunion in NOS depleted animals.
Disclosure: The authors declared no competing interests.

P55

Osteoblastic Micro-RNAs Regulate Cortical Bone Formation

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MicroRNAs are short (~25 nucleotides) RNAs that play a major role in the regulation of gene expression in many eukaryotic cellular processes. Many microRNAs have been reported to be involved in bone formation and remodelling. We have generated and characterised a new mouse strain (Dicerosx) that can be utilised to study the role of osteoblast specific microRNAs in skeletal maturation and fracture healing. It was generated by crossing Dicer flox/flox mice with OsxCreERT2 mice. Animal studies were approved by the Finnish Animal Ethics Committee. In this strain, by administering tamoxifen to Cre positive(OsxCreERT2-dicer floxed) mice, dicer can be inactivated in osterix expressing osteoblasts. Tamoxifen was given to young (3 weeks) and old (10 weeks) Cre positive and corresponding control mice for 3 days and they were followed for 5 weeks. The animals were then sacrificed and femura were analysed by MicroCT analysis. We found a significant reduction in the cortical bone volume and thickness in the Cre+ male and female mice, accompanied by reduced bone perimeter in the male mice, but not in female mice. We also studied the effects of Dicer inactivation in Osterix expressing osteoblasts during fracture healing. Under anaesthesia, closed tibial fractures were generated in Cre positive male mice as well as in Cre negative controls. Prior to the fracture, a small steel rod was inserted in the tibial shaft using surgical procedures. The animals were sacrificed at d14 and the calluses were analysed by MicroCT analysis. As compared with the controls, the total callus volume was decreased in the Cre positive animals. However, there was no significant difference in the amount of mineral deposited within the calluses. MicroRNAs appear to be important in the regulation of periosteal osteoblastic cells which was manifested as reduced cortical bone volume in the dicer inactivated animals.
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P56

Routine Blood-Samples as Predictors of Re-Operation due to Post-Operative Infection in Hip Fracture Patients

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Background: Several studies have shown an increased risk of both morbidity and mortality in hip fracture patients. There have been numerous studies focusing on different predictors of mortality. However, there is not much literature investigating postoperative complications and morbidity. The purpose of this study was to examine the relationship between in-hospital routine biochemical markers taken at admission and the risk of reoperation due to postoperative infection following a hip fracture.
Methods: A search on all hip fracture patients from Bispebjerg Hospital was conducted from the period October 2008 – June 2013, N=2,200. On this group of patients, a search for every reoperation caused by deep infection was conducted. Patients were included in the study if reoperation was within

3 months, resulting in 25 cases. For every case, two randomly chosen hip fracture patients from the hip fracture database from Bispebjerg Hospital matching in sex and age and without postoperative infection were found, resulting in 50 control patients. From the laboratory database blood-samples taken at admission were extracted.
Results: Two markers were found to have a statistically significant association with the risk of reoperation due to postoperative infection: CRP above 26 mg/l (OR 4.84 [1.69;13.87], p=0.002) and albumin lower than 35 g/l (OR 4.57 [1.64;12.73], p=0.003).
Conclusion: This study shows that CRP and albumin can be used for predicting the risk of reoperation due to postoperative infection in hip fracture patients.
Disclosure: The authors declared no competing interests.

P57
Biodegradable Magnesium-Silver-Alloy Implants Augment Fracture Callus Formation in Mice
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Implant removal after fracture healing is often indicated but requires a second surgery. Biodegradable implants might therefore be an attractive alternative to standard steel or titanium implants. We therefore developed an intramedullary nail made of a novel biodegradable magnesium-silver-alloy (Mg2Ag, 2.5% Ag) and tested its *in vivo* degradation, biocompatibility, and effect on fracture repair using a long-bone fracture model in mice. Briefly, Mg2Ag intramedullary nails of 0.8 mm diameter were introduced into the right femur of 8-weeks old C57Bl/6 male mice. Compared to steel nails and no implant controls, Mg2Ag implants degraded over time with remnants being still visible 100 days after implantation as determined by radiographs. Fracture healing was investigated using the same model after introducing open femoral midshaft fractures. Mice were overall healthy and no differences in body weight or any histological abnormalities in kidney, liver, muscle, or spleen were found. Radiographs, µCT, and bone histomorphometry revealed that compared with steel implants, fractures supported by Mg2Ag nails demonstrated a significant increase in callus size with a higher bone formation rate, number of osteoblasts and osteoid volume, while the number of osteoclasts, osteoclast surface, and the eroded surface were decreased. These findings indicate that intramedullary Mg2Ag nails may augment bone formation while reducing bone resorption, leading to a larger callus size. However, degradation of Mg2Ag implants causes hydrogen production. Indeed, gas bubbles were found in the soft tissue surrounding the fracture zone and within the femora, leading to misshaped bones. Our findings therefore suggest that Mg2Ag implants degrade in mouse long bones over time without systemic adverse effects and support callus formation while causing hydrogen production. Thus, additional investigations are needed to further determine the applicability of Mg2Ag alloys for fracture healing.
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P58
Adiponectin Deficiency in Female Mice Leads to Age-Dependent Changes in Bone
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Adiponectin, a peptide-hormone secreted from adipocytes, is involved in the regulation of energy homeostasis, glucose and lipid metabolism. Clinical studies show inverse relationships between circulating adiponectin concentrations and bone mineral density. Studies of the bone phenotype of adiponectin deficient mice (APN-KO) by different groups produced inconsistent results. The aim of our study was to conduct a systematic analysis of the bone phenotype of APN-KO mice, by adhering to strict experimental planning guidelines for *in vivo* studies. Groups of 10 WT C57Bl/6J and 10 APN-KO (C57Bl/6J background) female mice were culled at 8, 14, 21, 28 and 37 weeks of age. Bone architecture of femora was analysed by microCT (SkyScan 1172), and body composition was determined longitudinally by DEXA (Lunar PIXImus Densitometer). The WT and APN-KO animals had similar weights until week 37, when the WT mice were heavier (27.2±2.6g for WT and 24.63±1.9g for APN-KO, $P<0.05$). At 8 and 14 weeks, trabecular %BV/TV was lower in APN-KO mice (1.4-fold, $P<0.001$ and 1.3-fold, $p<0.05$ at 8 and 14 weeks, respectively), with no differences between the groups at later time points. Cortical %BV/TV was lower in the APN-KO groups at all the time points ($P<0.001$) and cortical thickness was lower from week 14 onwards ($P<0.01$). Analysis of body composition showed that from week 21 onwards, APN-KO animals had approximately 1.5-fold lower % fat ($P<0.0001$). Osteoclastogenesis assays in bone marrow cultures treated with $1,25(OH)_2D_3$ showed increased numbers of TRAP+ multinucleated cells in cultures from APN-KO mice (>1.5-fold increase, $P<0.01$). Our results show a consistent reduction in cortical volume and thickness and reduced trabecular volume in young animals. The contribution of the increased osteoclastogenesis measured *ex vivo* to the phenotype requires further investigation. Analysis of bone strength is underway to determine the functional consequences of the changes in bone architecture.
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P59
Does Bisphosphonates alter Bone Defect Healing?
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Systemic Pamidronate (APD) administration potentially modulates local bone cellular activities and accordingly alters bone

defect healing (intramembranous ossification). Metaphyseal tibia bone defects were created in 6-month-old female rats, which were injected with APD (0.1 mg/kg-5 d/w) or vehicle for 2 or 4 weeks. Bone samples were analysed by micro-computed tomography, histomorphometry and nano-indentation in metaphyseal 2nd spongiosa (MC), a region close to the defect (CDC), within the healing defect (DC) and in cortical defect bridging region. In cortical and trabecular healing zone, a blurry tetracycline labelling was observed at week-2 in both groups, indicating rapid bone formation while osteoid thickness and surfaces remained unaltered; interestingly bone formation was maintained in APD group. In contrast, bone formation rate in MC and CDC was decreased in APD group (~-60% each, p<.001) at the same time point. At week-4 osteoid surfaces and bone formation rate were lower in APD group independently of the trabecular sites while mineral apposition rate was also lower in APD group at cortical compartment. As expected, APD reduced active osteoclast surfaces at weeks-2 independently of the site (~-40%, p<.001). At weeks-4, bone resorption was even more decreased in all trabecular compartments (MC and CDC: -60%, p<.001, DC: -40%, p<.001) while it tended to be increased at the periost and decrease at endost. This resulted in higher trabeculare bone mass in APD group at the two time points and in a trabecularised cortical-shell in the APD group at week-4. Nano-indentation tests demonstrated an improvement of bone material level properties at week-4 in APD treated rats as evaluated at CDC (higher hardness) and cortical bridge (higher hardness and elasticity). Inside the healing zone, APD decreases resorption and maintained formation during bone modelling phase (i.e. first 2-weeks) leading to a positive bone balance, then inhibits bone remodelling and improves bone material level properties.

Disclosure: The authors declared no competing interests.

P60

Thyroid Hormone Interacts with the Sympathetic Nervous System, via Alpha$_{2A}$ Adrenergic Receptor, to Regulate the Longitudinal Bone Growth

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An important finding of the recent years is that bone remodelling is under control of the central nervous system (CNS), with the sympathetic nervous system (SNS) acting as the peripheral effector. Evidence suggests that the SNS negatively regulates bone mass via β_2-adrenergic receptor (AR-β_2), which is expressed in osteoblasts. However, previous research by our group has demonstrated that mice with double inactivation of α_{2A} and α_{2C} adrenergic receptors (α_{2A}/α_{2C}-AR$^{-/-}$) exhibit a high bone mass phenotype (HBM), in spite of presenting chronic sympathetic hyperactivity and intact β_2-AR. By immunohistochemistry, we showed that α_{2A} and α_{2C} adrenergic receptors (α_{2C}-AR and α_{2A}-AR) are expressed in osteoblasts, osteocytes, osteoclasts and in chondrocytes of the epiphyseal growth plate (EGP), of the secondary ossification centers and of the articular cartilage, which suggests that α_2 adrenergic receptors also mediate the actions of the SNS in the skeleton. We also found that α_{2A}/α_{2C}-AR$^{-/-}$ mice are resistant to the thyroid hormone (TH) excess-induced osteopenia and that mice with the single inactivation of α_{2A}-AR or α_{2C}-AR are resistant to the reduction of bone longitudinal growth caused by thyrotoxicosis. These findings suggest that TH interacts with the SNS, through α_2-AR signalling to regulate bone metabolism and growth. The present study aimed to investigate the role α_{2A}-AR and the possible interaction between the SNS and TH to regulate the longitudinal bone growth (LBG). Thus, we evaluated the bone growth and the morphology of the femoral EGP of 21-day old female wild-type (WT) and α_{2A}AR$^{-/-}$ mice (n=8/group), treated for four weeks with a supraphysiological dose of triiodothyronine (7µg/100gBW/day), to mimic hyperthyroidism (Hyper), or treated with inhibitory drugs of the thyroid function, methimazole (0.1%) and sodium perchlorate (1%), added to drinking water, for hypothyroidism (Hypo) induction. Compared with WT mice, euthyroid α_{2A}AR$^{-/-}$ mice showed lower femur length; an important disorganisation of the proliferative zone (PZ) with the presence of lacunae between the columns of chondrocytes; and a significant decrease (84%, p<0.001) in the thickness of the hypertrophic chondrocytes zone (HZ) of the EGP. In WT animals, Hypo promoted expected alterations: a significant reduction in the LBG, PZ disorganisation and a decreased thickness of HZ. Surprisingly, Hypo had no effect on the EGP of α_{2A}AR$^{-/-}$. On the other hand, Hyper caused an increase in the thickness of the reserve zone (RZ) in α_{2A}AR$^{-/-}$ mice, but not in WT mice. These findings support the hypothesis that the SNS regulates the LBG via α_{2A}AR and that a TH-SNS interaction can regulate this process.

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The Reborne Project: Regenerating Bone in Patients using Autologous Mesenchymal Stem Cells

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Bone is the most frequently transplanted tissue with about 1 million procedures annually in Europe. Autologous bone transplantation is the primary procedure for regenerating large bone defect in patients, but is limited in quantity and leads to complications at the second surgical site. Bone tissue engineering that associates mesenchymal stem cells (MSC), synthetic scaffolds and molecular signals may become an alternative to bone grafts in orthopaedic and maxillofacial surgery. The objectives of the European project REBORNE are to perform clinical trials using cultured cells and advanced biomaterials triggering bone healing in patients. Four clinical studies aiming at demonstrating the safety and efficacy of this advanced therapy medicinal products (ATMPs) have been approved in several European Countries and are currently underway. Preclinical studies that have supported the approval of these

trials will be presented. After receiving informed consent from patients, 30 ml of bone marrow was aspirated from the iliac crest. MSC were cultured in medium containing human platelet lysate. After 3 weeks of culture, cell therapy units were capable of producing about 400 million of clinical grade MSC. The culture conditions and quality controls were standardised to ensure the consistency of the ATMP across European blood transfusion centres. The optimal MSC dosage for bone formation was first determined by implantation of biphasic calcium phosphate (BCP) granules in ectopic sites of nude mice. MSC/BCP achieved ectopic bone formation with bone marrow territories. Cells of both host and human origin contributed to bone formation. The dose was then scaled up to clinically relevant sizes and transportation of fresh MSC was validated. Bioactivity and engraftment were also studied by bioluminescence imaging (BLI) of Luciferase expressing MSC and *in situ* hybridization using the human-specific repetitive Alu sequence. Critical sized bone defects were successfully repaired in skull of mice, femurs of rats and metatarsus of sheep using MSC and BCP. MSC were produced, transported, mixed with BCP biomaterial and implanted, mimicking the clinical scenario where large bone defects are regenerated. To date, 28 patients with non-union long bone fractures and 14 patients with osteonecrosis of the femoral head have been successfully treated in France, Spain, Germany and Italy.

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OSTEOGROW - BMP6 Device for Enhance Bone Healing

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High doses of BMPs are needed to achieve clinical success in spinal fusion and long bone non-union fractures with currently available devices. We found that BMP6 was less sensitive to endogenous inhibitors. Recently, we discovered that patient's blood coagulum could be used as a BMP6 carrier due to high binding affinity to specific blood components. The BMP6 carrier is a whole blood derived coagulum (WBCD) from the peripheral blood which acts as an endogenous biocompatible material (OSTEOGROW). More than 80% of BMP6 added to the full blood remains incorporated, bound mainly to its extracellular matrix components. Pharmacokinetic studies showed that BMP6 is rapidly cleared from the blood of mice and rats after IV dosing. Presence of BMP6 in circulation is minimal after systemic application and BMP6 is not distributed into the deep tissue compartment. No organ accumulation has been detected by immunohistochemistry. Release of BMP6 from the coagulum in *in vitro* conditions showed slow discharge from the coagulum with a mean residence time of approximately 7 days. In animal models the osteogenic biological activity of newly produced BMP6 was confirmed without inducing inflammation and oedema in the surrounding tissues. In a model of critical size defect of rabbit ulna WBCD containing BMP6 fully re-bridged the bone defect at a significantly accelerated rate as compared to commercial BMP7 containing bone device. We found that WBCD with 50µg of BMP6 compared to commercially used device with 3.5 mgs of BMP7 was 2 orders of magnitude more potent in *in vivo* rabbit ulna critical size defect. Clinical grade of BMP6 will be tested clinically in two indications for regeneration of the metaphyseal bone, compartments where BMP2 and BMP7 have not been effective. Safe, affordable and non-toxic BMP6 based autologous carrier OSTEOGROW will promote faster bone healing and reduce the need for secondary interventions.

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Delayed Healing in a Sheep Model of Osteoporosis Hallmarked by Persisting Cartilage and Increased Osteoid Formation

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Osteoporosis leads to increased fracture risk. Dysregulation of osteoblast and osteoclast lead to a high-turnover phenotype, which reduces bone mass and lowers bone mineral content. Therefore, osteoporotic fractures are a huge clinical challenge. This study utilised 32 female Marino sheep with an osteoporotic bone status to examine bone healing in the iliac crest drill-hole. Animals were divided into 4 groups: 1) non-operated control group (K group). 2) Bilaterally ovariectomised (O group). 3) Bilaterally ovariectomised and treated with special diet deficient of calcium and Vitamin D (OD group). 4) In addition to treatment in 3 this group received a biweekly dosage of glucocorticoid treatment (ODS group). Bone defects were 7.5 mm diameter 20 mm length and healing was examined at two time points (M=month) 5M and 8M. Histographs were evaluation using Movat pentachrom staining and quantified with ImageJ V 1.47 software. Statistical analysis ran in IBM SPSS V. 21. Qualitatively, K group showed cortical bridging by 5M and trabecular bone formation by 8M. However, treatment combinations affected cortical bone bridging and trabecular bone formation. Cartilaginous tissue was present after 8M mostly in OVXDS. Histomorphometrical analysis showed that healing progressed from 5M to 8M in the K group through higher total ossified tissue (TOT) and lower total cartilage tissue (TCT). TOT was lowest in the OVXDS compared to other groups. Further, OVXD group had higher osteoid portion when compared with all other groups. Nonetheless, OVXD and OVXDS showed cartilage remnant at 8M in contrast to both K and OVX groups. Osteoblast and osteoclast specific staining and molecular markers are currently being analysed to identify discrepancies in healing

regulation under the influence of estrogen deficiency, malnutrition and glucocorticoid treatment.
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Potential Effects of Strontium Ranelate on Bone Mineralisation and Implant Osseointegration
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Strontium ranelate is a drug that has promising effects on the treatment of women with post-menopausal osteoporosis. An advantage of this drug compared to others is its unique effect on bone cells: it simultaneously promotes bone formation by osteoblasts and inhibits bone resorption by osteoclasts. However, its effects on the mineralised bone matrix are not yet fully known. In particular, due to the presence of strontium ions (Sr^{2+}) in its composition, this drug could lead to several changes in the bone mineral phase, which must be evaluated. Besides the treatment of osteoporosis, strontium ranelate was also shown to increase the osseointegration of bone implants in animals. The better understanding of how the drug promotes this effect, and mainly of its actions on the cellular component of the bone/implant interaction, may contribute to its possible application aiming to increase the clinical success of bone implants. The main goal of this study was to evaluate effects of strontium ranelate on bone mineralisation and on the interaction of osteoblasts with titanium surfaces. In particular, we evaluated, in osteoblast cultures treated with strontium ranelate: (1) the formation and nature of the mineralised matrix; (2) the composition and crystal structure of the mineral phase; and (3) the cell behaviour and matrix formation on titanium surfaces. As main results, we noticed that strontium ranelate: (1) promotes bone mineralisation preserving the overall nature of the matrix; (2) leads to several changes in intrinsic properties of the mineral phase, such as: substitution of slightly less than 10% of Ca^{2+} by Sr^{2+} in the apatite crystal lattice, increase in the lattice parameters *a* and *c*, increase in the type-B CO_3^{2-} content, and changes in PO_4^{3-} environments; and (3) has positive effects on the interaction of osteoblasts with titanium surfaces, including in cell proliferation, differentiation, and formation of bone-like matrix.
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P65
Screening Osteogenic Properties of Follistatin, Nell1 and CCN2 on Osteoprogenitor Cells *In Vitro*
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Treatment of large bone defects in the craniomaxillofacial or axial skeleton is a challenging process. The gold standard treatment involving harvesting of bone from another anatomical location in the patient, can lead to donor site morbidity, increased pain cost and recovery time. The ideal therapy would use cell free materials to fill the defect combined with chemical agents that induce and instruct bone defect repair by cells of the patient. This would avoid the costly stages of cell harvesting and preparation under Good Manufacturing Practice conditions associated with cell therapy based approaches. Here we describe the assessment of the ability of 3 proteins, Follistatin, Nell1 and Connective Tissue Growth Factor (CTGF/CCN2) to induce osteogenic differentiation and migration of osteoprogenitors and mesenchymal stem cells (MSCs) as a model for the recruitment of resident cells in the *in vivo* situation. Cells were cultured with addition of each factor at a range of doses (Follistatin: 0.8 nM, 2 nM and 5 nM, Nell1: 10 ng/ml, 100 ng/ml and 500 ng/ml and CCN2: 10 ng/ml, 50 ng/ml and 100 ng/ml). svHFO (simian virus Human Foetal Osteoblasts) preosteoblasts and MSCs were cultured in osteogenic differentiation medium (dex glycerophosphate etc) for up to 21 days. Alkaline phosphatase activity (ALP), calcium deposition, total protein and DNA were measured. Chemotaxis towards each factor was also measured. Follistatin induced a dose dependent increase in ALP activity and mineralisation (calcium accumulation). In contrast, there was no significant difference in any osteogenic parameter measured with the addition of Nell 1 or CCN2. This work indicates the potential of follistatin to be used as a pro-osteogenic factor for the induction of bone formation for the repair of large bone defects.
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Effect of the Functional Appliance on the Mandible Growth in Rats
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In growing patients with a retrusive mandible, growth modification is the main goal of orthodontic treatment. Orthopaedic forces to the TMJ used in functional appliances might influence the interaction between the mandibular condyle and the glenoid fossa. Histological and biochemical research in this field mainly aims to provide basic information about the nature of the skeletal growth modification in response to functional appliance therapy. The aim of this study was to develop a simple, convenient, and functional animal model to reproduce the orthodontic effects of functional appliances. The appliance was mounted onto upper jaw of

5 week-old Sprague-Dawley rats and 3, 7, 14, 21, and 28 days onward, animals were sacrificed for the analysis. Metric analysis was carried out by measurements from micro-CT taken from sacrificed animals. Condyle width was increased initially after mounting of functional appliance but was not changed afterwards. Condyle was observed to position more to the anterior than control 3 days after functional appliance application, but relocated to the similar position as control with time. Mandibular length was increased by 7 days after functional appliance application than control, but the difference was gradually diminished with time. Mandibular height was steadily increased after functional appliance application than control. Based on these results, comparison between effects of animal model and clinical cases were carried out and showed that prognosis of experimental functional appliances were similar to that of previous clinical studies. These results suggested that this experimental animal model could provide a reliable and testable way to elucidate the important histological and biochemical changes induced by clinical functional appliance.
Disclosure: The authors declared no competing interests.

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Micro Computed-Tomography and Biomechanical Testing Analysis Show Decreased Fracture Healing in Leptin-Deficient Mice which does not Increase after Controlled Cortical Impact Injury
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Background: It is clinically observed that patients suffering from traumatic brain injury (TBI) can show increased callus formation in long-bone fractures. The underlying mechanisms of this phenomenon, however, are poorly understood. In a first experimental study we could reproduce this effect in wild-type mice. Leptin acts as a regulator of bone growth in the central nervous system. The aim of the present study was to assess fracture healing in leptin-deficient mice and to measure the impact of an additional TBI.
Method: 138 female 12 weeks old B6.V-Lep-ob/JRj mice (Janvier Labs, Saint Berthevin, France) (body weight 50,67 g ± 3,04 g) were divided into 4 groups: Control (n = 28), fracture (n = 37), TBI (n = 35), combined trauma (TBI + fracture) (n = 38). Fractures were stabilised using an external fixator (RISystem, Davos, Switzerland) at the left femur, the osteotomy gap was sawed at 0.7 mm. The TBI was induced at the left cortex with a controlled cortical impact injury (CCI). Postoperative in-vivo micro-computed tomography scans (callus volume, callus density) were performed weekly. Using frontal and sagittal reconstructions, the bridging of the osteotomy gap was also evaluated with a scoring system. After 3 and 4 weeks the animals were sacrificed and biomechanical testing (max. torque, max. stiffness) was performed.
Results: Micro-computed tomography showed no significant differences between the fracture and combined trauma group regarding callus volume and callus density. The CT scoring system revealed non-union rates of 93% and 96% at 3 weeks in the fracture and combined trauma group respectively. Also, the biomechanical analysis could not show significant differences in max. torque and max. stiffness between fracture and combined trauma group.
Conclusion: In this study we could show that fracture healing is impaired in leptin-deficient mice. Furthermore, TBI does not have an impact on fracture healing in mice in the absence of leptin. Given that wild-type mice respond to TBI with significantly increased callus formation, leptin seems to play an important role as regulator of the TBI-driven accelerated fracture healing.
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Cortical Bone Response Following Implantation: Changes in Bone Remodelling and Mechanical Regulation on Periosteal and Endosteal Surfaces
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Successful fracture fixation in osteoporotic individuals requires a better understanding of the interplay between implant insertion, bone remodelling and mechanical regulation. Here, we characterise the mechanical regulation of cortical bone remodelling following implantation. We performed ovariectomy on C57BL/6 mice (OVX, n=9) to induce bone loss, whereas in SHM (n=8) the ovaries were not removed. Implants were inserted into the sixth caudal vertebrae and the bone-implant system was scanned weekly using *in vivo* micro-computed tomography for 6 weeks. Cortical bone was divided into a peri-implant region (close to the implant) and a reference region (far from the implant). Periosteal and endosteal bone remodeling were assessed by overlapping consecutive scans. Micro-finite element models were generated to compute the strain energy density (SED) at formed, resorbed, and quiescent sites. Following implantation, peri-implant bone formation rate was transiently increased on periosteal and endosteal surfaces in SHM and OVX. However, on endosteal surface, bone resorption rate (BRR) in SHM was elevated soon after implantation while in OVX higher values were measured at later time points; periosteal BRR was significantly increased only in SHM. Even in the presence of the implant, periosteal bone remodelling was mechanically regulated as shown by SED at formed/resorbed sites being higher/lower than at quiescent sites. On endosteal surface far from the implant OVX showed a prevailing bone resorption independently of SED, while close to the implant the normal mechanical regulation pattern was detected. Our results indicated an initial fast peri-implant bone forming phase, in which formation and resorption were strongly mechanically coupled only in SHM. Endosteal remodeling seems to be less mechanically controlled than periosteal remodeling and implant insertion restored locally the mechanical control also on endosteal bone. Our approach may be used to develop smart implants

attaining long-term stability by manipulating the remodeling process to favour bone formation over resorption.
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Secretome of Human Dental Pulp Stem Cells (Dpscs): Differential Gene Expression During Induction of Osteogenic/Odontogenic and Chondrogenic Differentiation

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Constant remodelling of extracellular matrix (ECM) is a hallmark during physiological conditions, such as stem cell differentiation, embryogenesis and tissue repair. MMPs, MMP-inhibitors (TIMPs and RECK) and MMP-inducer (EMMPRIN) play a key role in these processes, being part of cell proteolytic secretome. Transforming Growth Factor Beta superfamily members, such as TGF-β and BMPs, are responsible for bone, tooth and cartilage formation and modulate the expression of molecules involved on matrix turnover. In this way, we evaluated gene expression of MMPs, TIMPs, RECK and EMMPRIN during induction of osteogenic/odontogenic and chondrogenic differentiation from DPSCs *in vitro* by qPCR. DPSCs were isolated from extracted human third molars and grown in clonogenic medium (α-MEM medium + 10% FBS + 100 μM ascorbate) and differentiation induction in presence of osteogenic medium (10 mM β-glycerophosphate, 1 μM dexamethasone and 100 μM ascorbate), odontogenic medium (10 mM β-glycerophosphate, 1 μM dexamethasone and 100 μM ascorbate + 50 ng/mL BMP-7) or chondrogenic medium (DMEM/F12 + 500 ng/mL insulin + 1 μM dexamethasone + 50 μM ascorbate + 10 ng/mL TGF-β1 + 1mM sodium pyruvate) for 35-days. For each differentiation, a differential gene expression pattern was observed and our results suggest that both TGF-β1 and BMP-7 may regulate MMPs, their inhibitors and inducer gene expression during osteogenic/odontogenic and chondrogenic differentiation *in vitro* from DPSCs.
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Structure of Lower Incisor in Rats of Different Ages After 60-Day Inhalation Of Epichlorohydrin

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Background: The aim was to study structure of lower incisor in rats of different ages after 60-day inhalation of epichlorohydrin vapours (EV) and administration of thiotriazoline (Th) and *Echinaceae tinctura* (ET) as medication.
Methods: The study involved 420 male rats of three ages. The animals were split into the groups: 1st group comprised control animals, the 2nd group comprised the animals that received inhalations of EV in dosage of 10 MPC as a single 5-hour exposure per day, 2rd group - inhalations of EV and intraperitoneal Th in dosage of 117.4 mg per kg, 4th group - inhalations of EV and intragastric *ET* in dosage of 0.1 mg of active substance per 100 grams of body weight. After EV discontinue cross-sections of the lower incisor sampled as a segment next to the second molar tooth were HE stained. Morphometry included measurements of odontoblast layer, pre-dentin, and mature dentin and mesio-distal size (V. Luzin, 2011).
Results: By the 1st day upon EV discontinue, width of odontoblasts layer, pre-dentin layer, dentin layer and mesio-distal size in young rats were lower than that of the controls by 12.83%, 11.35%, 11.47% and 10.70%. In mature rats all the values listed were lower by 12.56%, 9.74%, 9.56%, and in old rats – 8.85%, 8.67%, 7.37% and 8.95%. In readaptation period after EV discontinue, alterations gradually reduced in young and mature animals yet by the 60th day majority of the values were markedly lower than those of the controls and old animals yielded few signs of restoration. After administration of Th, restoration of dentin structures in young rats was registered from the 1st to the 30th day, in adult rats – from the 1st to the 60th day, and in old rats - from the 15th to the 60th day. After administration of *ET,* positive effects in young and adult rats were observed from the 7th to the 60th day and in old rats – from the 15th to the 60th day.
Conclusions: 60-day inhalation of EV results in inhibition of functional activities of dentin secreting structures of the lower incisor. Restoration of dentin structure well depended on age of animals. Young animals exhibited faster restoration while in old animals such manifestations were scarce. Administration of Th or *ET* resulted in restoration of structure of the lower incisor. Th appeared to be more effective than *ET.*
Disclosure: The authors declared no competing interests.

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Microfluidic-Based Co-Cultures: an Open Door to Bone Innervation Studies

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Mineralised tissues were demonstrated to be highly innervated by sensory neuronal population, which is one of the main peripheral regulators for bone development and mass accrual. Therefore, particular interest has been shown in the involvement of sensorial neuropeptides in the regulation of bone tissue due to the effect of soluble factors, produced by nerve fibres, associated with changes in the activity of bone cells. Nonetheless, the intercommunication between the bone and peripheral nervous system is scarcely understood. Therefore, given the importance of nerve fibres in bone microenvironment and the lack of adequate tools to study such crosstalk, we

used the compartmentalised microfluidic platforms to recapitulate the *in vivo* bone sensorial innervation. Microfluidic compartmentalised devices allow the separation of the somal and axonal fraction of neurons and, more interestingly, the separation of different cell types. We have benefit from these proprieties and developed microfluidics-based co-culture systems comprising dorsal root ganglion and osteoblasts, in 2D/3D chemically functionalised hydrogels, to mimic the properties of naturally occurring tissue extracellular matrices providing more accurate mechanical and biochemical signalling factors. Additionally, we have also developed a computational application of a mathematical algorithm, available online for the scientific community, which improved the system's readout by enabling the automatic quantification of neurite outgrowth within non-neuronal cells in these platforms. Together the entire system emerges as a potential new tool to be explored for modelling of innervation processes and pharmacological screening with an automated and non-subjective tool for the system readout. We believe that this system can be further translated to study the innervation of different peripheral tissues in physiological or pathological conditions.

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Early Changes in Micro-Architectural Bone Parameters in the Development of Complex Regional Pain Syndrome Type 1 after a Distal Radius Fracture

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Complex regional pain syndrome type 1 (CRPS-1) is a clinical syndrome characterised by pain that is disproportionate to the inciting event (e.g. a fracture), oedema, decreased function, changes in skin colour and bone loss. Although Südeck proposed an inflammatory origin over a century ago, the pathophysiology is still unknown. Current hypotheses include neurological, vasomotor and immune dysfunctions. In two recently published papers we reported that the healing process of stable distal radius fractures can be assessed using high-resolution peripheral quantitative computed tomography (HR-pQCT) in combination with finite element analysis (FEA). A 54-year old postmenopausal patient who was included in this study developed CRPS-1 during fracture healing. HR-pQCT scans of the fractured wrist of this patient were performed at 12, 23, 45 and 86 days and 26 months post-fracture according to the study protocol and showed a remarkable decrease in trabecular density, first detected six weeks post-fracture (fifth percentile), along with a decrease in trabecular number, which persisted at 26 months. There was an initial temporary strong increase in markers of bone formation and resorption. Surprisingly, the coronal cross-sections revealed dramatic resorption of trabecular bone proximal, but not distal of the fracture line. In contrast, the cortical region healed normally as the fracture gaps were bridged and cortical thickness increased. This resulted in restoration of bone stiffness as estimated with FEA at 26 months similar to patients without CRPS-1. These results, though from a single patient, illustrate the possible role of HR-pQCT in the early identification of CRPS-1 related bone loss and could identify patients at risk for developing CRPS-1. The anatomical localisation of trabecular bone changes in the diaphysis and proximal of the fracture site suggests involvement of the vasculature of the nutrient artery in the resorption process.

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The use of Bone Anabolic Factors for Improving Tendon-Bone Regeneration

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Background: Healing of hard-soft tissue interfaces are a significant clinical problem. The tendon-bone interface is a particular problem due to the high levels of strain it is exposed to. Furthermore, there are currently no accepted biological treatments for improving the healing of this complicated tissue interface. Parathyroid hormone (PTH) is an anabolic factor used clinically to enhance bone formation. It increases insulin growth factor-1 expression (a factor important in tendon healing), and has recently been shown to enhance tendon healing in a model of flexor tendon injury, and tendon-bone healing in a model of anterior cruciate ligament reconstruction. Lactoferrin is a pleiotropic growth factor with anabolic effects on bone and cartilage; both important constituents of the tendon-bone interface, where tendons insert into distinct layers of cartilage and finally bone. Here, we aim to elucidate the effects of PTH and lactoferrin on tendon cell biology.

Methods: Primary tenocytes were harvested from rat tails and treated with a range of concentrations of PTH and bovine lactoferrin (bLF). Cell growth was determined using alamarBlue® assays and collagen deposition was measured by Sirius red

dye release. Tenocyte differentiation was assessed by measuring gene expression levels using real-time PCR.
Results: Neither PTH nor bLF had an effect on tenocyte growth or collagen production. Similarly, bLF had no effect on the expression on genes important in tendon biology, such as scleraxis and tenomodulin, but did increase the expression of alkaline phosphatase. PTH decreased scleraxis expression, aggrecan and sox9 expression and increased alkaline phosphatase expression.
Conclusion: These results indicate that both PTH and lactoferrin are likely to induce tenocytes to trans-differentiate away from a tendon-specific lineage, suggesting that while they may not be ideal for tendon regeneration, they may have use in the healing of tendon-bone interfaces where tendon inserts into cartilage, and then bone.
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P74

Pro- and Anti-Inflammatory Cytokines Differentially Affect the Expression of Osteocalcin by Mesenchymal Stem Cells

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Inflammatory cytokines present during bone healing have been suggested to enhance osteogenic differentiation of mesenchymal stem cells (MSCs). However, little is known about the effects of anti-inflammatory cytokines (e.g. IL-4) on MSCs differentiation, although these are also produced during the bone healing process. We hypothesised that both pro- and anti-inflammatory cytokines enhance osteogenic differentiation of MSCs. Human adipose tissue-derived mesenchymal stem cells (hASCs) were cultured in αMEM containing 2% platelet-lysate, 50 μM ascorbic acid-2-phosphate, 5 mM β-glycerophosphate and 10 nM 1,25-$(OH)_2$ vitamin D_3. TNF-α, IL-6, IL-8, IL-17F and IL-4 (10 ng/mL) were present during the first 72h of culture. Gene expression was quantified by real-time quantitative PCR. Significant effects ($P<0.05$) are mentioned below. TNF-α decreased expression of osteogenic differentiation markers RUNX2 (6-fold), ALP (2-fold), and COL1 (4-fold) at day 4. IL-6 increased expression of the proliferation marker Ki67 (2-fold) at 6 h, but decreased expression of COL1 (2-fold) at 48h and RUNX2 (6-fold) at day 4. IL-8 decreased expression of OCN (5-fold) at 48h, and expression of RUNX2 (4-fold) at day 4. Similarly, IL-17F decreased expression of RUNX2 by 4-fold at day 4. IL-17F increased expression of OCN (3-fold), albeit only at 6h. Finally, IL-4 decreased expression of RUNX2 at day 4 (10-fold) and day 7 (6-fold). In contrast to the other cytokines, IL-4 enhanced expression of OCN at day 4 (3-fold) and day 7 (5-fold). Our findings suggest that pro-inflammatory cytokines TNF-α, IL-6, IL-8, and IL-17F, and the anti-inflammatory cytokine IL-4, exert different effects on the expression of osteogenic markers by hASCs. The pro-inflammatory cytokines are unlikely to enhance osteogenic differentiation of MSCs, but IL-4 might, since it enhanced expression of the late osteogenic marker osteocalcin. The modulation of inflammation after bone fracture may be useful to enhance bone repair in patients with inflammatory diseases or for tissue engineering strategies.
Disclosure: The authors declared no competing interests.

P75

Defective Skeletal Mineralisation in PiT2/Slc20a2-Deficient Mice

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Skeletal mineralisation is a process of fundamental importance to all vertebrate animal species. During skeletal growth and remodelling, calcium and phosphate (Pi) are required for the formation of biological apatites. The rate at which mineralisation occurs is dependent, in part, on the local availability of Pi and calcium. Although implication of Pi transporters in mineralisation process appears evident, the identity of this (or these) protein(s) is yet to be determined. In vertebrae, the type III cotransporters (*PiT1* and *PiT2*) are the only NaPi cotransporters identified so far in skeletal tissues, and are thus considered as essential suppliers of Pi for skeletal mineralisation, despite the absence of *in vivo* evidence. This putative role in bone mineralisation was also derived from *in vitro* studies that have showed that *PiT1*, but not *PiT2*, expression is regulated by factors regulating bone cells. However, we recently demonstrated that PiT1 hypomorphic adult mice have a normal bone mineralisation. To assess the role of PiT2 in bone development, growth and mineralisation, we underwent the phenotypic characterisation of *PiT2* knockout mice (french ethical approval #02286.01). We show that *PiT2*$^{-/-}$ mice are subviable and that 50% of the *PiT2*$^{-/-}$mice are dying off around birth. After birth, we observe that *PiT2*$^{-/-}$ mice are growth retarded and exhibit impaired skeletal mineralisation. Quantitative faxitron analyses show lower bone mineral content and weaker and less stiff bones in *PiT2*$^{-/-}$ mice compared with controls. Analyses of histological sections of the upper tibia from *PiT2*$^{-/-}$ mice show a decreased bone formation and also a reduced growth-plate mineralisation at postnatal day 16. We are now investigating expression of key regulators of Pi homeostasis. To this aim, we are performing conventional biochemical analyses and RT-qPCR from bone, kidney, gut and blood samples. Altogether, these data suggest that PiT2 is a key sodium-phosphate cotransporter for skeletal mineralisation.
Disclosure: The authors declared no competing interests.

P76

Manipulating Time and Space to Achieve Endochondral Bone Formation

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Autologous bone grafts are the gold standard treatment for large bone defects but the available quantify of these grafts is limited, highlighting the need for alternative graft sources. Endochondral bone formation can be achieved by chondrogenically priming mesenchymal stem cell (MSC) pellets *in vitro* and implanting *in vivo*. In this study we investigated the effects of shorter *in vitro* priming on chondrogenesis and subsequent *in vivo* bone formation. We also characteriaed a novel micropellet (µpellet) construct to possibly enhance *in vivo* bone formation. Chondrogenically differentiated MSC pellets and µpellets were encapsulated using alginate or fibrin and cultured for 2, 5, 7, 10, 14, or 28 days. Gene expression was analysed and safranin O staining was performed. Due to consistent *ColII* and *ColX* upregulation and positive GAG staining after 7 days of culture, this time point was chosen for further *in vivo* study. µpellet-fibrin constructs cultured *in vitro* for 7 and 28 days retain a similar chondrogenic potential and showed upregulation of hypertrophic genes including *ColX* and *BMP2*. Fibrin constructs were subcutaneously implanted in athymic nude mice for 8 weeks. Similar volumes of calcified tissue between µpellet and pellet conditions were found in *ex vivo* µCT scans. Bone and bone marrow were found in both pellet and µpellet-fibrin constructs after either 7 or 28 days of *in vitro* priming. Here we have shown that bone formation can be achieved after only 7 days of *in vitro* chondrogenic priming. Interestingly, µpellet constructs formed comparable quantities of bone like tissue to pellets and show upregulation of hypertrophic genes *in vitro* when encapsulated in fibrin, indicating µpellet-fibrin constructs could possibly be cultured for an even shorter period *in vitro* and form bone *in vivo*. With the proper optimisation these µpellets could also be utilised as an injectable bone graft for future regenerative medicine applications.
Disclosure: The authors declared no competing interests.

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Computational Solution "ODR-ATA" to Measurement of Bone Density from Radiographic Density

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The radiographic densitometry is the technic that allows the evaluation of bone density, which uses references created in inert material (e.g. aluminium) especially to the investigation of bone modifications and illnesses such as osteoporosis. To increase technical precision some authors suggest the use of mathematical equations, such as the least squares method, Laplace´s theorem, and the Rule of Sarrus allowing better results when achieving the adjustment of the curve that is characteristic of X-ray absorption by what is being studied. The usage of technologies such as Java, gnuplot, broffice calc, all of open code, allowed us to develop the software "ODR-ATA", which is capable of analysing and processing digital radiographic images and of calculating values of density close to the values of the object under study. Recently, various paid solutions have the support to perform radiographic densitometry. The purpose of this study is to develop a computational solution of free code to calculate bone density starting from the radiographic densitometry. In order to test the software, I performed an experiment that started with the radiography of an aluminium ladder of unknown values. I performed 10 repetitions and evaluated steps 4,5,6,7 and 8 of the aluminium ladder. The results I achieved with "ODR-ATA" for steps of number 5,6,7 and 8 show a precision of 3.64%% and 3.63%, respectively, which attest for the validity and reliability of the software in the discovery of the bone density obtained through the radiographic densitometry.
Disclosure: The authors declared no competing interests.

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Osteogenic Markers in Early Alveolar Bone Formation

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Alveolar bone is an important part of the dynamic tooth-bone complex. In the case of mouse first molar, the alveolar bone becomes morphologically apparent at the embryonic (E) day 14. Gradually, the bone encapsulates the tooth germ and the next process, bone remodelling, starts to accommodate the growing tooth germ and create the final support during and after eruption. This worked aimed to search for candidates in osteogenic networks around the start of the alveolar bone formation. In the first part of research, stages E13, E14 and E15 were compared regarding expression of osteogenic genes using the Mouse Osteogenic PCRArray. Along with collagens, altered gene expression was observed in the case of *Fgf3, Ctsk, Icam-1, Mmp9, Itga3 and Tuft1* where significant increase was demonstrated. Decrease or additional subtle changes were found also for other genes. The next step of investigation was focused on localisation of proteins corresponding to genes with increased expression within the mandibular/alveolar bone using immunohistochemistry. The findings were correlated with cell types and morphogenetic events in early alveolar bone formation.
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CALCIOTROPIC AND PHOSPHOTROPIC HORMONES AND MINERAL

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Circulating FGF23 is Elevated In Acute Myocardial Infarction

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Myocardial infarction (MI) is a major cause of death worldwide. Epidemiological studies have linked vitamin D deficiency to MI incidence and support a role of vitamin D signalling in the pathophysiology of MI. However, the mechanisms by which vitamin D may regulate cardiovascular function in MI are still controversial. Because fibroblast growth factor-23 (FGF23) is a master regulator of vitamin D hormone production, and has been shown to be associated with cardiac hypertrophy per se, we explored the hypothesis that FGF23 may be a previously unrecognised pathophysiological factor causally linked to progression of cardiac dysfunction post-MI. To test our hypothesis, we induced experimental MI in two different rat and mouse animal models by permanent or transient ligation of the left descending coronary artery. Animals without ligation served as sham-operated controls. We show that circulating intact Fgf23 was profoundly elevated, whereas serum vitamin D hormone levels were suppressed after MI in rat and mouse models. Western blotting and immunohistochemical analysis revealed that both skeletal and cardiac expression of Fgf23 was increased after MI. Serum parathyroid hormone, phosphate, sodium, and calcium remained unchanged in MI animals. Since it has been suggested that the FGF23 co-receptor Klotho may have an FGF23 independent protective role in the regulation of cardiac function we measured the abundance of soluble Klotho by western blotting. However, serum Klotho remained unchanged in MI rats and mice. In conclusion, our study has uncovered that experimental MI causes a profound up-regulation in intact circulating Fgf23, independent of changes in serum soluble Klotho or serum parathyroid hormone. Although the molecular link between the cardiac lesion and circulating Fgf23 concentrations remains to be identified, our study has uncovered a novel heart – bone – kidney axis which may have important clinical implications, and may inaugurate the new field of cardio-osteology.

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***Klotho* Null Foetuses Reveal that FGF23 does not Regulate Foetal-Placental Phosphorus Homeostasis or Prenatal Bone Formation and Mineralisation**

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Fibroblast growth factor-23 (FGF23) regulates serum phosphorus by acting on kidneys to excrete phosphorus and reduce calcitriol. Absence of FGF23 causes severely disturbed phosphorus and bone metabolism. In contrast, *Fgf23* null foetuses at embryonic day (ED) 17.5 and 18.5 had normal phosphorus and skeletal parameters. In order to rule out that another phosphate-regulating FGF compensates for loss of FGF23 *in utero*, we studied the effect of loss of the FGF23 co-receptor Klotho, which causes a phenotype similar to loss of FGF23 in postnatal mice and humans. We mated *Klotho*$^{+/-}$ mice and studied their WT, *Klotho*$^{+/-}$, and *Klotho* null foetuses. On ED 17.5, amniotic fluid (largely urine) was collected and placental ^{32}P transport at 5 min was measured. Whole bodies at ED 18.5 were reduced to ash and the mineral content was assayed. At ED 18.5, serum and tibial sections were obtained. qPCR was done on ED 18.5 placentas and kidneys. Intact FGF23 was measured by EIA (Kainos). There was no alteration is foetal serum phosphorus (3.04±0.05 mM in WT, 3.01±0.06 mM in null), amniotic fluid phosphorus (1.75±0.15 mM in WT, 1.83±0.13 mM in null), placental ^{32}P transport (90±9% in WT, 89±11% in null), serum FGF23 (107±14 pg/mL in WT, 138±14 pg/mL in null), or serum calcium and amniotic fluid calcium. *Klotho* nulls had normal tibial lengths, morphology, and mineralisation; and whole body skeletal ash weight and content of calcium, phosphorus, and magnesium. qPCR of placentas and kidneys confirmed absence of *Klotho* in the nulls but normal expression of *Cyp27b1*, *Cyp24a1*, *Fgf23*, *Fgfr1-4*, and *NaPi2a,b&c*. In conclusion, since loss of FGF23 or Klotho have no effect, FGF23 is clearly not a regulator of foetal-placental phosphorous and bone metabolism. Active transplacental delivery of phosphorus does not require FGF23 or Klotho, and supersedes FGF23's potential effects on phosphate handling by foetal kidneys.

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Long-Term Effects of Recombinant Human Parathyroid Hormone, rhPTH(1-84), on Bone Remodelling in Patients with Hypoparathyroidism: 3-Year Data from the Open-Label RACE Study

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Deficient parathyroid hormone, the hallmark of hypoparathyroidism, can lead to low bone turnover and increased bone mineral density (BMD). In the pivotal phase III, placebo-controlled REPLACE study and the subsequent dose-blinded RELAY study, treatment with rhPTH(1-84) restored mineral homeostasis and increased bone turnover markers (BTMs) in patients with hypoparathyroidism. In RACE, an open-label extension trial of REPLACE and RELAY, patients

initiated treatment with subcutaneous rhPTH(1-84) at 25 or 50 µg/day, with possible up-titration to 50, 75, or 100 µg/day if active vitamin D and oral calcium could be further reduced. Serial measurements of serum calcium and phosphorus were performed, and serum BTMs were analysed, including BSAP, CTX, and P1NP. BMD was measured annually by dual-energy X-ray absorptiometry. All data presented as mean ± SD. This analysis included 39 patients (79% female) who received at least 36 months of rhPTH(1-84) therapy. Hypoparathyroidism duration was 16±13 years. At baseline, optimised albumin-corrected serum calcium and phosphorus levels were 2.1±0.2 and 1.6±0.2 mmol/L, respectively. At month 36, serum calcium was maintained at 2.1±0.2 mmol/L, whereas serum phosphorus decreased to 1.4±0.2 mmol/L. Baseline BTMs were low-normal (BSAP, 9.3±3.2 µg/L; CTX, 206.9±171.3 ng/L; P1NP, 32.3±18.3 µg/L). Actual BTM levels increased at month 36 (BSAP, 15.6±9.4 µg/L; CTX, 480.3±457.1 ng/L; P1NP, 129.7±114.7 µg/L). A trend toward BMD Z-score reduction was observed at all sites except lumbar spine (change from baseline at month 36: total hip, –0.05±0.51; femoral neck, –0.05±0.61; distal one-third radius, –0.44±0.76; lumbar spine, 0.24±0.84). Adverse events were similar to those in the REPLACE study. Long-term treatment of patients with rhPTH(1-84) was associated with continued improvement in bone and mineral homeostasis, evidenced by increases in BTMs, reductions in most BMD Z-scores, and improved serum calcium and phosphorus levels. The results provide further evidence for the clinical utility and efficacy of rhPTH(1-84) in the treatment of hypoparathyroidism.

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P83

Expression Profile of NGF-BDNF and Osteocalcin Genes in Brain, Bone, Fat Stores and Reproductive Organs

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Bone mass, metabolism and reproduction are regulated coordinately. The bone-derived osteocalcin (Ost) favours insulin sensitivity, male fertility and neurogenesis. The neurotrophins BDNF and NGF are involved in energy and bone metabolism. NGF regulates fertility elevating LH in the female, Ost-/- mice show obesity and high LH in spite of decreased testosterone. To investigate the NGF-osteocalcin interaction we analysed by RT-PCR the mRNA levels of NGF, BDNF, Ost. and their receptors p75NTR/NTRK1, TRKb and Gprc6a, respectively in adipose WAT/BAT, reproductive organs, brain and bone (positive controls) of 3 months old female and male mice. The mRNA levels of NGF and p75NTR are 50% higher in BAT than the brain. NGF and its receptors are down-regulated in WAT and bone in both genders. Osteocalcin and Gprc6a are up-regulated in bone and brain, downregulated in BAT/WAT. BDNF and TRKb expression in bone is higher than brain, but lower in BAT/WAT; TRKb is down-regulated in bone and up-regulated in adipose tissue. NGF is up-regulated in the ovaries/uterus, but down-regulated in the testes. The mRNA levels of p75NTR is, respectively, 300%, 100% and 50% higher in testis, ovaries and uterus than the brain. NTRK1 is down-regulated in all tissues. The Gprc6a is expressed in the testes, not in ovaries and uterus. BDNF and TRKb are down-regulated in the sexual organs. Therefore, the up-regulation of NGF and related-receptors in fat is consistent with NGF as an energy regulator. The inverse correlation of NGF and BDNF in fat and bone, shows these exerting opposite effects on leptin with BDNF regulating bone. The up-regulation of p75NTR in the testes match the Gprc6a expression, and may be responsible for higher LH in the Ost-/- mice. The animal care was performed in accordance with the DIRECTIVE 2010/63/EU. The protocol was approved by the Ethics Committee of the University of Bari, Italy.

Disclosure: The authors declared no competing interests.

P84

Prevalence of Primary Hyperparathyroidism in Patients with Prostate Cancer

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The prevalence of primary hyperparathyroidism (PHPT) in patients with prostate cancer has not been investigated. We consecutively enrolled 69 men (aged 51-87) with histologically confirmed prostate cancer (31 were candidate to radical prostatectomy, radiotherapy or hormone therapy, HT; 7 were in active surveillance and 31 already had radical prostatectomy while on HT). Eight patients had skeletal metastases. After clinical evaluation, we measured serum PSA (total and ratio), chromogranin A, S-100, ionised calcium, parathyroid hormone, 25(OH)D, bone alkaline phosphatase, collagen type I C-terminal cross-linked telopeptide, intact and C terminal fibroblast growth factor (FGF) 23. The protocol was approved by the "Sapienza" University of Rome Ethics Committee. We found a high prevalence of PHPT among cancer patients (n=9, 13%). We stratified the population on the basis of Gleason Score (GS) [≤7(3+4) and ≥7(4+3)], PSA (<4 ng/ml, between 4 and 10, and >10) and HT (yes/no) and used a generalised linear model with a logit link to predict the probability of developing PHPT. The model showed that only GS, C-terminal FGF-23 and HT had a significant effect ($p<0.05$). After controlling for other variables, we observed that the rise in FGF-23 increases the odds of developing PHPT by 2% ($p<0.02$), and higher values of GS are associated with a higher probability of developing PHPT (log-odds=3.6, $p<0.01$). Conversely, higher GS in association with HT play a significant protective role ($p<0.01$), decreasing the odds

of developing PHPT by 8%. The prospective investigation shows a remarkable prevalence of PHPT in men with prostate cancer. The multivariate analysis demonstrates that more aggressive prostate cancer, as determined by GS, is a significant predictor of increased risk of PHPT, suggesting a possible causative relationship rather than a simple association between the two disorders. A thorough biochemical evaluation of mineral metabolism parameters is therefore mandatory in patients with prostate cancer.
Disclosure: The authors declared no competing interests.

P85
Decreased Vitamin D Metabolites and Increased Vitamin D Binding Protein (DBP) and Parathyroid Hormone (PTH) Concentrations in Obese Premenopausal Finnish Women
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Recent studies have reported lower serum 25-hydroxyvitamin D (S-25OHD) concentrations in obese subjects compared with normal weight individuals. However, differences in DBP or in free and bioavailable S-25(OH)D concentrations between obese and normal-weight persons are not so well studied. We compared 43 obese (35.7±4.3 kg/m^2) and 144 normal-weight (22.5±1.6 kg/m^2) women aged 37-47. Blood samples and data on vitamin D and calcium intake (from food and supplements) were collected as well as lifestyle habits. Comparisons between the normal-weight and obese subjects were made with ANCOVA. DBP concentrations were significantly higher in obese compared with normal-weight subjects: (388 ± 89 mg/l and 358±88 mg/l, respectively; p= 0.033). Total S-25(OH)D concentrations were 56.7±18.2 nmol/l in normal-weight subjects and 50.4±17.9 nmol/l in obese subjects; p=0.004.The concentration of calculated free 25OHD was lower in obese woman compared with normal-weight women (10.7±4.1 nmol/l and 12.6± 4.3 nmol/l, respectively; p=0.002). Also the calculated bioavailable 25(OH)D (free+albumin bound) was lower in obese persons (p<0.001). Total vitamin D intake, S-PTH concentration and sunny holidays were used as covariates. S-PTH was higher in obese compared to normal weight subjects (65.1±29.9 and 54.0±23.5 ng/l, respectively; p=0.017).Total vitamin D intake was higher in normal-weight than in obese subjects (15 µg/day and 11µg/day, respectively; p=0.012), which in both groups met the vitamin D recommendation in the Nordic European countries (10 µg/day). The observed associations between BMI and total- free-and bioavailable 25(OH)D, DBP and PTH suggests that obese individuals may differ in their vitamin D metabolism and may be at higher risk of having vitamin D deficiency.
Disclosure: The authors declared no competing interests.

P86
***In Vitro* Apatite Formation from Synthetic Polyphosphate and Alkaline Phosphatase**
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Biological nucleation of apatite mineral involves a local increase in calcium and/or phosphate concentration. In 1923, Robison postulated that alkaline phosphatase (ALP) activity resulted in an increased local inorganic phosphate (Pi) concentration. In the presence of calcium, carbonate and at neutral pH, this biochemically controlled increase in Pi concentration could provide the chemical potential to nucleate a carbonated apatite. The substrate for ALP that results in skeletal apatite nucleation has not yet been identified. The hypotheses that a calcium-carbonate-polyphosphate species is a substrate for ALP, and that an apatite mineral is a possible product of ALP activity was tested. Amorphous materials produced by mixing synthetic sodium polyphosphate with solutions containing calcium, magnesium and carbonate with a final total P (as polyphosphate) concentration of 0.5 M resulted in a hydrogel. Similar precipitations with sodium phosphate instead of sodium polyphosphate resulted in mineral precipitation. The hydrogel or Pi precipitate were filtered and incubated in a background electrolyte buffered at pH 9 and 37°C to optimise ALP activity. After 1 week, the precipitates were characterised by powder X-ray diffraction and Raman spectroscopy. Initial and final solution pH and phosphate concentration (by colorimetry) were measured. Raman spectroscopy identified a Pi peak with a Raman shift of ~960 cm^{-1} for precipitates formed from Pi, and from polyphosphate incubated with ALP. Precipitates incubated without ALP maintained a distinct polyphosphate shift at ~1170 cm^{-1}. Powder X-ray diffraction patterns from precipitates formed in the presence of ALP exhibited many peaks, including peaks characteristic of carbonated apatite, among other peaks. Colorimetric analysis for phosphate indicated an increase in Pi concentration over the initial buffer solutions, which contained no Pi. This represents preliminary *in vitro* evidence that the combination of polyphosphate anions as the sole phosphorus source, and active ALP can result in apatite mineral nucleation.
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P87
Direct Systemic Effects of BMP 2 and 7 on the Skeleton
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BMP2 and 7 are used locally in FDA approved indications including complicated long bone fractures, non-unions, and spinal fusions. It is unknown whether their systemic release following a local implantation might impact systemically the bone metabolism. Furthermore, it is unknown whether systemic BMP effects on bone are direct or mediated by

calciotropic hormones. To answer these questions we examined effects of systemically administered BMP2 and 7 on bone in a newly developed rat model with a low level of calciotropic hormones. Removal of thyroid and parathyroid glands (TPTx) in rats resulted in the decreased level of thyroid hormones, PTH, calcitonin and $1,25(OH)_2D_3$ and a subsequent bone loss assessed by microCT and measurement of serum bone formation and resorption markers, including osteocalcin, C-telopeptide, osteoprotegerin and receptor activator of nuclear factor kappa-B ligand. Additionally, BMP2 and 7 were tested *in vitro* to estimate their influence on osteoblast and osteoclast activity. The administered doses have been calculated according to published bioavailability data from pre-clinical BMP2 and 7 studies. TPTx resulted in bone loss, which was restored by systemic administration of 10-70 µg/kg of BMP2 and all doses of BMP7. BMP2 showed a higher capacity for enhancing trabecular microarchitecture, i.e. increasing trabecular number and diminishing trabecular spacing. In contrast, BMP7 augmented trabecular thickness. *In vitro* experiments revealed that BMP2 and 7, when uncoupled, increased the number and activity of both osteoblasts and osteoclasts. Collectively, both BMP2 and 7 showed ability to increase bone volume in an *in vivo* environment of low calciotropic hormones. Therefore, locally administered BMP2 and 7 from bone devices might become partially available in circulation but will not mediate a systemic bone loss.

Disclosure: The authors declared no competing interests.

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Serum Vitamin D, Parathyroid Hormone and Calcium are Associated with Non-Vertebral Fracture Independent of Cortical Bone Architecture

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Background: Vitamin D deficiency and hyperparathyroidism is associated with bone loss. However, it is less clear whether serum levels of Vitamin D, parathyroid hormone (PTH) and calcium are associated with fracture risk, and if so, whether it is mediated through increasing cortical porosity and thickness. We tested whether lower vitamin D and higher PTH and calcium levels were associated with fracture independent of these cortical features.

Methods: We measured serum 25 (OH) Vitamin D, PTH and total calcium, femoral neck areal bone mineral density (FN aBMD) using densitometry (DXA), and femoral subtrochanteric cortical porosity, cortical thickness, and trabecular bone volume/tissue volume (BV/TV) using StrAx1.0 software in 211 postmenopausal women aged 54-94 years with non-vertebral fractures and 232 controls.

Results: Women with fracture exhibited lower serum levels of vitamin D (76.4 vs. 82.9 nmol/L), higher PTH (4.58 vs. 4.13 pmol/L), and total calcium (2.43 vs. 2.35 mmol/L) than controls, higher porosity of the total cortex (43.8 vs. 41.7%), compact cortex (35.3 vs. 34.3%), transitional zone (59.3 vs. 58.4%), and reduced cortical thickness (4.06 vs. 4.36 mm), $p < 0.05$, but not lower trabecular BV/TV (0.266 vs. 0.272%), $p = 0.81$. Each standard deviation (SD) decrease in vitamin D and each SD increase in PTH and calcium was associated with increased odds for fracture (OR 1.31 95% confidence interval (CI) (1.07-1.61), 1.28 (1.04-1.58) and 1.78 (1.41-2.24), $p < 0.05$, respectively after adjustment for age, height, weight, femoral subtrochanteric cortical porosity and thickness. Higher calcium levels, but not vitamin D and PTH remained associated with fracture after mutual adjustment, even after additional adjustment for FN aBMD (OR 1.85; 1.45-2.35, $p < 0.001$).

Conclusion: Calciotropic hormones are associated with increased odds for fracture, independent of cortical bone architecture. These findings are suggesting that calciotropic hormones may increase the risk of fracture through other mechanisms.

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P89

Effects of Long-Term Vitamin D3 Deficiency on Mouse Bone Structure

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Background: Vitamin D3 is a key regulator in calcium homeostasis. Vitamin D3 deficiency is associated with mineralisation defects and increased fracture risk and is common among elderly. Whereas short-term vitamin D3 deficiency in young animals is a widely studied phenomenon, there are relatively few *in vivo* experiments to investigate the effects of long-term vitamin D3 deficiency on bone tissue in older animals. The objective is to characterise the effect of long-term adult vitamin D3 deficiency *in vivo* on bone histomorphometric parameters in ageing mice.

Methods: Mice were exposed to either a vitamin D3 adequate diet (n=7) containing 1 IU/g Vitamin D3 or a vitamin D3 deficient diet (n=8) containing 0 IU/g Vitamin D3 from 10 months until 24 months of age. Both diets contained 0.5% calcium. Serum 25(OH)D levels were assessed by LC/MS MS. Using Goldner- and TRAP-stained undecalcified bone sections of the proximal humerus, bone volume (BV/TV), bone surface (BS), osteoclast surface (Oc.S/BS), trabecular diameter (Tb.Dm), trabecular number (Tb.N) and trabecular separation (Tb.Sp) were measured. Differences between groups were statistically tested with the Student-t test.

Results: Average serum 25-hydroxyvitamin D3 levels in control mice were 55.7 nmol/l, all deficient mice had 25-hydroxyvitamin D3 levels <4 nmol/l. No significant differences in bone structural variables between the vitamin

Table 1 [P89]

	BV/TV	Tb.Dm (mm)	Tb.N (per mm)	Tb.Sp (mm)
Control group (mean [SD])	10,17% [2,38%]	0,055 [0,010]	6,59 [0,58]	0,099 [0,008]
Vitamin D3 deficient group (mean [SD])	8,57% [2,15%]	0,051 [0,004]	6,50 [0,51]	0,105[0,014]
Student T-test	0,195	0,280	0,765	0,339

D3 deficient and control mice were found. Mineralisation defects were not apparent on qualitative inspection in the vitamin D deficient mice. (See Table 1.)
Conclusions: Despite the long term vitamin D3 deficiency, the bone volume and structure was not different in long-term vitamin D3 deficient mice compared to control mice. Bone resorption was similar in both groups. The sufficient calcium in the diet could possibly have reversed the effects of vitamin D3 deficiency.
Disclosure: The authors declared no competing interests.

P90

The Effects of Long-Term Adult Vitamin D Deficiency on the Regulation of CYP27B1 in Mouse Bone

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Vitamin D deficiency, a common condition in the elderly population, causes a decrease of calcium absorption from the intestine and secondary hyperparathyroidism which leads to bone loss, osteoporosis and mineralisation defects in the long term. Long-term vitamin D deficiency may also affect the regulation of the 1α-hydroxylase gene CYP27B1 in bone, since bone cells are able to synthesise 1,25-dihydroxyvitamin D_3 needed for stimulation of osteoblast differentiation and mineralisation. Therefore, the aim of this study is to determine the effects of long-term adult vitamin D deficiency on bone mineral density and the regulation of CYP27B1 in mouse bone. Ten-month-old male mice were fed a diet containing 0.5% calcium and 0 (n=8) or 1 (n=9) IU/gram vitamin D_3 for 14 months. At an age of 24 months, the mice were sacrificed for mRNA analysis of CYP27B1, CYP24 and VDR from tibiae and kidneys using RT-PCR and for micro-computed tomography analysis of the humeri. Plasma 25-hydroxyvitamin D_3 ($25(OH)D_3$) levels were measured using liquid chromatography-tandem mass spectrometry. Mean $25(OH)D_3$ plasma levels were <4 nmol/L in the vitamin D deficient mice and 57.4 nmol/L in the control mice. Bone mineral density was not different between the vitamin D deficient and control mice. Moreover, the humeral cortices showed similar patterns of bone mineral density in both groups. CYP27B1 and VDR mRNA levels in bone and kidney did not show differences between the vitamin D deficient and control mice. Kidney CYP24 mRNA levels were similar in both groups, while bone CYP24 mRNA levels were not expressed. In conclusion, the diet contained probably sufficient calcium to maintain an adequate bone mineralization degree after long-term vitamin D deficiency. Interestingly, although plasma $25(OH)D_3$ levels were very low and thus almost no substrate was present, bone cells continued to express CYP27B1 at the same level.
Disclosure: The authors declared no competing interests.

P91

Correlations of Serum 25(OH) Vitamin D Levels With the Calcium-Phosphate Homeostasis and Bone Mineral Density in Type 2 Diabetes Patients on Oral Antidiabetic Drugs – Preliminary Data

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Background: Vitamin D regulates calcium and phosphate absorption in the small intestine as well as bone mineralisation. Our objectives were to describe the correlations of serum 25(OH)D with the serum and urinary calcium, phosphate and creatinine, the serum iPTH and β-crosslinks, as well as with the bone density (BMD) in type 2 diabetes patients on oral antidiabetic drugs.
Methods: One hundred type 2 diabetes patients participated – 56 men and 44 women. The mean age and diabetes duration of the women was 59 and 9.8 years, of the men – 58 and 7.7 years, respectively. None of them was taking calcium / vitamin D supplements or antiresorptive treatment. Serum levels of 25(OH)D were measured as 25(OH)D Total, iPTH by an electro-hemi-luminescent method and β-crosslinks by ECLIA (all three on an Elecsys 2010 analyzer, Roche Diagnostics, Switzerland); serum and urinary calcium, phosphate and creatinine - on a Cobas Integra analyzer. Hip BMD was measured on a GE Lunar Prodigy Pro bone densitometer (GE Healthcare, Madison, WI). Correlation analysis was performed on a SPSS 13.0 for Windows platform (SPSS Corp., Chicago, IL) and included 10 curves. The data were first analysed for the group as a whole, then separately for men and women and for vitamin D tertiles.
Results: Serum 25(OH)D was correlated with serum calcium and phosphate (R^2=0.417 and R^2=0.389, p<0.001), as well as with urinary calcium (R^2=0.091, p=0.03), but not with iPTH, urinary phosphate, creatinine or β-crosslinks. The correlation with BMD was of borderline significance. The sub-analyses according to gender or 25(OH)D tertiles did not add useful information.
Conclusion: In type 2 diabetes serum vitamin D has an impact on serum calcium and phosphate but not on their urinary excretion. iPTH, β-crosslinks and BMD are subject

to more complex regulation and the relative contribution of vitamin D is hard to prove.
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Analysis of Association between Genetic Variants of PTH SNPs and Serum 25(OH)-vitamin D
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Background: Parathyroid hormone (PTH) plays a crucial role in calcium metabolism and skeletal development, to some extent via altering vitamin D level, and hypersecretion of PTH is implicated in the aetiology of osteoporosis. In this study we analysed association between variants of promoter region of PTH gene and circulating 25-hydroxy-vitamin D (25(OH)D) level.
Methods: Circulating level of 25(OH)D and genotypes of PTH SNPs rs1459015, rs10500783 and rs10500784 were determined in healthy adults (N=386) of different nationalities living in Riyadh. Association between the different PTH genetic variants and corresponding mean serum 25(OH)D levels were statistically analysed.
Results: We observed high prevalence of vitamin D deficiency (<50 nmol/l) among all nationals living in Riyadh and the extent of deficiency ranged from 59% of Indians to 82% of Yemeni. Comparison of the means of 25(OH)D levels corresponding to different genotypes of PTH SNPs indicated that the T allele of SNP rs1459015 was significantly associated with higher level of 25(OH)D in the Sudanese (p=0.03), while the T allele of SNP rs10500783 was associated with higher level of 25(OH)D in Saudis (p=0.03). Analysis of different genotypes of the PTH SNPs indicated that carriers of the CC genotype of SNP rs1459015 had a higher risk of suffering from vitamin D deficiency in the Sudanese (p=0.02).
Conclusions: Specific variants of PTH SNPs were associated with altered levels of 25(OH)D and different degrees of vitamin D deficiency. Replication of these results in future studies may elucidate of the role of PTH in the regulation of vitamin D absorption and metabolism.
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P93
Tumour-Induced Osteomalacia due to a Gluteal Tumour: a Case Report
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Tumour-induced osteomalacia (TIO) is a rare paraneoplastic process, characterised by poorly-healing fragility fractures and proximal myopathy. Inappropriately elevated levels of FGF-23 secreted from an occult mesenchymal tumour, lead to renal phosphate wasting, hypophsophataemia, decreased 1,25-vitamin D levels and subsequent fractures. Treatment is centred on localisation and removal of the causative tumour, which often achieves symptomatic and biochemical resolution. We report a case of a 47-year-old man with tumour-induced osteomalacia (TIO) from a benign tumour in the gluteus minimus muscle. Our case highlights the importance of measuring and acting upon serum phosphate in patients with fragility fractures, proximal myopathy and/or bone pain.
Disclosure: The authors declared no competing interests.

CANCER AND BONE: BASIC, TRANSLATIONAL AND CLINICAL

P94
Targeting Sclerostin Prevents Bone Loss in a Murine Model of Multiple Myeloma
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Multiple myeloma (MM) is a neoplastic disease of plasma cells associated with a progressive and highly destructive osteolytic bone disease, severe bone pain and pathological fractures. The bone disease is a result of both increased bone resorption and decreased bone formation. Anti-resorptive agents do limit fractures, however, the disease often progresses and no approaches exist to repair bone lesions. The Wnt/β-catenin pathway plays a critical role in the regulation of bone formation. Soluble wnt antagonists such as Dkk1 and Sclerostin, inhibit bone formation. Anti-Dkk1 treatment improves outcomes in pre-clinical models of MM, and is under clinical trials in MM patients. The role of sclerostin in MM is however unknown. C57BLKalwRij mice were injected with 5TGM1eGFP murine myeloma cells or saline (Naïve) and treated with anti-sost (weekly, 100 mg/kg) or control antibody (cont-Ab), until harvest day on 21. FACS analysis revealed no difference in tumour burden with anti-sost treatment. MicroCT analysis demonstrated that 5TGM1eGFP caused an 11% reduction in cortical bone volume and a 27% reduction in cancellous bone volume/total volume (BV/TV) ($p<0.01$). Anti-sost treatment prevented this bone loss, increasing cortical bone volume by 20% and cancellous BV/TV by 26% compared with cont-Ab treated tumour bearing mice ($p<0.05$). This was such that cortical BV and cancellous BV/TV were equivalent to the naïve cont-ab group. Taken together, these data suggest that sclerostin has a role in multiple myeloma induced bone loss. Further analysis of bone cell activity will confirm the mechanism of bone loss prevention was through enhanced bone formation, increasing the thickness of cortical and cancellous bone structures. Anti-sost is in clinical trials for the treatment of bone loss in osteoporotic patients. Our data suggest a potential new application for anti-sost treatment in patients with MM.
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P95

The KISS1 Receptor – a New Player in the Tumour Microenvironment of Multiple Myeloma

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Multiple myeloma (MM) is a haematological disease characterised by an aberrant proliferation of plasma cells within the bone marrow. As a consequence, 90% of all patients suffer from osteolytic bone loss, in turn promoting tumour growth in a "vicious cycle". Of importance for disease progression is the direct cell-cell interaction between MM cells and the mesenchymal stem cells (MSCs)/osteogenic precursor cells (OPCs) residing within the tumour niche. To detect myeloma cell-specific interaction molecules on bone forming cells, we performed global gene expression analyses for MSCs and OPCs after myeloma cell contact. Whole genome analyses were performed using Affymetrix gene chip HG-U133 2.0 Plus Array and RNA (each n=5) from MSCs and OPCs that had been co-cultured for one day with the plasmacytoma cell line INA-6. Differentially expressed genes included angiogenic factors such as VEGF-A, and the adhesion molecule ICAM-1, both of which are of importance in MM disease progression. In addition, we identified the G-protein coupled receptor KISS1 receptor (KISS1R) as a gene upregulated in MSCs as well as OPCs after direct contact with INA-6 cells. Transwell experiments revealed that this upregulation is predominantly dependent on direct cell-cell contact. Upregulation was further confirmed at protein level using the fluorescently labelled ligand KISS1 (KISS1-Alexa 633) and a second, murine MM cell line. Furthermore, western blot analyses showed MM cell line-dependent expression of KISS1 as well as KISS1R. Treatment with KISS1 ligands Kp-10 and Kp-54 as well as a KISS1R antagonist had no impact on INA-6 cell or MSC proliferation. First experiments using KISS1-Alexa 633 in a murine model of MM bone disease showed an accumulation of fluorescence in tumour-burdened limbs compared with contralateral controls. These data suggest that the KISS1/KISS1R system may play a role in the cross-talk between MM and bone-forming cells thereby allowing the imaging of myeloma bone disease.

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Bisphosphonates and Skeletal Metastasis: Resorption Agonists Reduce Zoledronic Acid's Effectiveness in Bone-Tumour Microenvironment by Modulating PTP Expression in Osteoclasts

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Nitrogen containing bisphosphonates, *e.g.* Zoledronic acid (ZOL), are currently used in oncological practice to reduce skeletal complications with bone metastasis of several neoplasms. Bisphosphonates (BP) inhibit bone resorption by inducing apoptosis in osteoclasts (OC). Several studies and meta-analyses report no overall beneficial effect of prescribing ZOL for specific breast and prostate cancer treatments owing to treatment failures and development of resistance towards ZOL. The bone-tumour microenvironment is a complex niche due to the presence of numerous bone resorption agonists (RAs), secreted by tumour cells. The effects of these RAs on osteoclast differentiation/function in the presence of BP are unknown. Our overall objective is to investigate the mechanisms of resistance developed by osteoclasts to overcome the therapeutic effects of ZOL in tumour microenvironments simulated using an *in vitro* model. OC were prepared using human peripheral blood derived mononuclear cells and the effect of ZOL and conditioned media from oral squamous cell carcinoma cell line OSCC 12 (OSCC-CM) on OC differentiation and function were examined. Quantification of TRAcP positive OC revealed a 33% reduction in OC number by ZOL (10 nM) when treated alone for 10 days, but ZOL efficacy is reduced to ½ when cultured along with OSCC-CM (20%). qPCR analyses done following 48 h treatment with ZOL +/-OSCC-CM revealed that non-receptor type protein tyrosine phosphatase (PTP), PTPN13/cyt-PTPe, expression levels were reduced by 6-fold in with ZOL treatment. In contrast, there was a 3-fold increase of PTPN13 levels with ZOL+OSCC CM treatment when compared with ZOL alone. *In vitro* inhibition of PTPs using Orthovanadate (10 μM) for 24 h made PTPN13 susceptible to ZOL-induced effects. Based on these results, we propose that sustained PTP elevation in OC is responsible for reduced efficacy of ZOL. PTP activation is known to up regulate anti-apoptotic, pro-survival pathways. Thus simultaneous inhibition of PTPs in osteoclasts would help overcome the development of ZOL resistance. Small molecule PTP inhibitors should be evaluated to be developed as adjuncts to ZOL therapies and would be predicted to improve the outcomes of BP treatment in tumour microenvironments.

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Roundabout Receptors: a New Molecular Target for the Prevention of Bone Metastasis

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Roundabout receptors regulate axon guidance. A comparative transcriptomic analysis demonstrated that Robo1 and Robo4 receptors were overexpressed in a subpopulation of MDA-MB-231 breast cancer cells that only metastasise to bone (referred to as B02). Interestingly, high Robo4 expression in primary tumours from patients with breast cancer correlated with poor prognosis and increased risk of relapse to bone. *In vivo*, a 50% reduction in tumour burden was observed when Robo4-depleted cells were inoculated in the mammary fat pad of mice. Moreover, the extent of osteolytic lesions after injection of tumour cells into the caudal artery of mice was decreased in animals bearing Robo4-depleted tumours compared with animals bearing Robo1-depleted tumours. *In vitro*, the treatment of parental B02 cells with anti-Robo4 antibody dose-dependently inhibited B02 cell invasion. Altogether these results let us hypothesise that Robo4 might facilitate the engraftment of tumour cells in the bone marrow. To address this question, B02 cells were incubated with an anti-Robo4 antibody 1h before intra-tibial inoculation. The bone marrow was flushed from the hind limbs of animals 14 days after tumour cell inoculation. Flushed bone marrow cells were then cultured under antibiotic selection, enabling the selective growth of antibiotic-resistant tumour cells. The incidence of tumour cell colony formation was reduced by 50% when B02 cells were incubated with the anti-Robo4 antibody. *In vitro*, the co-culture of B02 cells with MC3T3-E1 osteoblastic cells generated an increase in Slit2 production, the Robo receptor ligand, by MC3T3 cells. Further, the treatment with anti-Robo4 antibody dramatically reduced B02 cell adhesion to MC3T3-E1 cells. These results provide strong evidence that axon guidance receptors Robo1 and Robo4 are involved in bone metastasis formation and that the use of an antibody directed against Robo4 receptor could lead to the development of innovative therapies to prevent metastatic niche formation in the bone marrow.

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P98

ALK1Fc Suppresses Tumour Growth by Inhibiting Angiogenesis and Notch Signalling in Human Prostate Cancer

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Prostate cancer is the second most common cancer in men worldwide. Lethality is almost inevitable due to the consequences of metastasis. Therefore, understanding the molecular pathways that underlie the emergence and spread of metastases from primary tumours are of great biological and clinical value. Our studies focus on the role of activin receptor–like kinase-1 (ALK1), a key regulator of tumour angiogenesis. ALK1 is a type I receptor in the transforming growth factor-β (TGFβ) superfamily with high affinity for its ligands Bone morphogenetic protein (BMP) 9 and 10 inducing phosphorylation of Smad1 and/or Smad5. Interestingly, its effect can be mediated also by ALK2/Smad1/Smad4 pathway. We employed a soluble chimeric protein (ALK1Fc) containing the human ALK1 extracellular domain that binds with high affinity BMP9 and BMP10, thus, preventing activation of endogenous ALK1, ALK2 and downstream signalling. Here we show for the first time in prostate cancer that ALK1Fc reduces BMP9- mediated signalling and decreases proliferation *in vitro* by disrupting the induction of Notch signalling, another major key pathway involved in prostate cancer development, progression and bone metastasis. In line with these observations we further demonstrate that ALK1Fc reduced tumour growth by impairing angiogenesis and affecting proliferation of human prostate cancer cells *in vivo.* Taken together this data suggest BMP9 as a possible therapeutic target and thus justifies the continued clinical development of drugs blocking ALK1 and ALK2 receptor activity.

Disclosure: The authors declared no competing interests.

P99

Liposomal Delivery of the Glucocorticoid Dexamethasone Inhibits the Growth of Malignant Bone Lesions from Human Prostate Cancer

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Metastatic bone disease is a detrimental stage in prostate cancer for which no satisfying treatment options exist

to date. Prostate cancer progression is strongly promoted by tumour-associated inflammation in which tumour-associated macrophages (TAM) play a prominent role by the secretion of growth-, inflammatory- and angiogenic factors. Angiogenic factors induce tumour angiogenesis and these chaotically organised blood vessels are typically characterised by enhanced vascular leakage. This characteristic can be exploited by the use of nanomedicinal drug delivery systems (i.e. liposomes). The long circulation time of liposomes, in combination with the leaky tumour vasculature, leads to efficient localisation and retention of these nanoparticles at the metastatic microenvironment, which is also referred to as the enhanced permeability and retention (EPR)-effect. After extravasation to the tumour microenvironment, liposomes are typically taken up by pro-inflammatory TAM. In this study, we evaluated the antitumor efficacy of liposomal encapsulated dexamethasone, a glucocorticoid receptor-agonist with strong anti-inflammatory activity, in a preclinical *in vivo* model for prostate cancer bone metastasis (intra-tibial growth of luciferase-expressing PC-3M-Pro4 cells in 6-week old male Balb-c nu/nu mice). We found that liposomes localise efficiently at malignant bone lesions. Intravenous administration of both free- and liposomal dexamethasone displayed potent antitumour efficacy, suggesting that dampening the tumour-associated inflammation, presumably via silencing of pro-inflammatory TAM, leads to inhibition of tumour growth. Interestingly, we found that the liposomal formulation of dexamethasone significantly outperforms administration of free (non-encapsulated) dexamethasone. It is important to note that liposomal dexamethasone was found to be well-tolerated at therapeutically-active dosages in both tumour-bearing mice and healthy CD Sprague Dawley rats. Taken together, our findings warrant further clinical evaluation of liposomal dexamethasone in patients with advanced, metastatic, prostate cancer. Phase I clinical trials are now in preparation.

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Genetic Variation is Involved in Impairment of Bone Mineral Density in Long-Term Adult Survivors of Childhood Cancer

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Background: Despite similarities in upfront treatment, impairment of bone mineral density (BMD) varies in long-term adult survivors of childhood cancer (CCS). We studied for the first time whether genetic variation is involved in impairment of BMD in adult long-term CCS.

Method: This cross-sectional single-center cohort study included 334 CCS (median follow-up time: 15.2 years [range 5.1-39.8]; median age at follow-up: 26.1 years [range 18.1-49.3]). Total body BMD (BMD_{TB}) and lumbar spine BMD (BMD_{LS}) were measured by dual-X-ray absorptiometry(DXA), and BMD was expressed as age-matched and gender-matched standard deviation scores (SDS;Z-score). We selected 12 candidate single nucleotide polymorphisms(SNPs) in 11 genes based on results of previous studies in the healthy population (*COL1A1, TNFSF11, TNFRSF11, TNRFSA11B, VDR, ESR1, WLS, LRP5, MTHFR, MTRR, IL6*). Multivariate analyses included apart from candidate SNPs, patient and treatment characteristics that were univariatly associated with BMD values.

Results: Multivariate analyses revealed that lower $BMD_{TB/LS}$ was associated with lower weight at follow-up ($p<0.01$) and BMD_{TB} with previously administered radiotherapy ($p=0.01$). Survivors with the homozygous minor allele (CC) genotype of rs2504063 (in *ESR1*: oestrogen receptor type 1) had a lower BMD_{TB}(-1.17 vs. -0.84; $p=0.01$) than those with the TT/CT genotype, but BMD_{LS} was not altered. Carriers of the homozygous minor allele (CC) genotype of rs599083 (*LRP5*: low-density lipoprotein receptor) revealed lower BMD_{TB} (-1.18 vs. -083; $p=0.04$), and lower BMD_{LS} values (-0.97 vs. -0.54; $p=0.02$) than those with the TT/CT genotype.

Conclusions: We showed that CCS who are carriers of candidate SNPs in the *ESR1* or *LRP5* genes are more vulnerable to loss of bone mass at an early adult age. Information on genetic factors variation in individual patients may be helpful in identifying survivors who are at risk for low bone density after childhood cancer treatment in addition to patient and treatment related factors.

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Tumour-Derived Alkaline Phosphatase Promotes Epithelial-Mesenchymal Transition (EMT) and Cell Survival in Bone Metastatic Prostate Cancer: a Novel Role in Prostate Cancer Metastasis

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Prostate cancer (PCa) bone metastases are associated with mixed osteolytic/osteoblastic lesions. Identifying novel mechanisms that underlie this debilitating bone disease will ultimately reveal new therapeutic targets. Here, we identified differential expression of the key osteoblastic enzyme alkaline phosphatase (ALP) in a panel of PCa cell lines with increasing metastatic potential and aimed to elucidate ALP function

and regulation in PCa *in vitro* and *in vivo*. ALP activity and mRNA expression were higher in osteoblastic ARCaPM cells than non-metastatic ARCaPE ($p<0.001$), and associated with *in vitro* mineralisation of PCa cells, indicative of functional osteomimicry. ALP expression was lower in osteolytic PC3 cells, and undetectable in most non-bone metastatic cell lines. ALP knockdown in ARCaPM cells resulted in ~50% decrease in cell viability and Mesenchymal-Epithelial Transition (MET), evidenced by a change in morphology, increased E-cadherin ($p<0.001$) and decreased migration. ALP knockdown in PC3 cells decreased cell viability ($p<0.01$). Pharmacological inhibition of ALP dose-dependently inhibited cell viability in a panel of bone metastatic PCa cells. Knockdown of the mesenchymal marker Snail, or treatment with extracellular ATP, a known growth inhibitor in PCa, resulted in ~40% decrease in ALP activity and expression ($p<0.05$) and induced MET, evidenced by a morphology change and altered expression of epithelial and mesenchymal markers. To investigate tumour-derived ALP *in vivo*, we analysed human primary and metastatic PCa samples from the MSKCC PCa dataset, demonstrating that ALP correlated positively with EMT markers (including Snail, $\rho=0.5827$, $r=0.5120$) and negatively with markers of tumour suppression (including PTEN, $\rho=-0.5487$, $r=-0.3338$) ($p<0.001$). Our study defines a novel mechanism whereby tumour-derived ALP induces EMT and survival of PCa cells, with ALP expression regulated by Snail and purinergic signalling. Correlation of ALP expression with known cancer genes demonstrates its importance in human disease. Hence, this study identifies a previously unsuspected role for tumour-derived ALP in PCa.

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P102

Dormant Myeloma Cells Express a Unique Transcriptome Profile: Identification of New Therapeutic Targets

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Multiple myeloma is a haematological disease that develops in the skeleton. The bone microenvironment supports myeloma growth and long-term survival of dormant myeloma cells. Despite new treatments, patients typically relapse, suggesting we need a better understanding of the regulation of dormant cells and their role in drug resistance. We developed a new approach to identify dormant myeloma cells *in vivo* and define the transcriptome profile of these cells in order to identify pathways that control dormancy. 5TGM1eGFP myeloma cells were labelled with the fluorescent membrane dye DiD. Dividing cells share DiD with daughter cells and become DiD^{neg}, whereas, non-dividing dormant cells retain DiD (DiD^{high}). DiD-labelled cells were injected (i.v.) into C57BLKalwRij mice and DiD^{high} and DiD^{neg} populations isolated by FACS at 7, 14, 21, or 28 days. Transcript profiling of each population was performed using mouse ST 2.0 whole genome arrays. DiD^{high} dormant cells were identified in bone marrow across all time-points. DiD^{neg} cells were first detected on day 14 and increased up to day 28. Over 300 genes were up-regulated and >300 genes were down-regulated in DiD^{high} cells compared with DiD^{neg} cells at day 28, (fold change >2, Q value <0.05). Gene set enrichment analysis (GSEA) confirmed the dormant status of DiD^{high} cells, with down-regulation of gene sets involved in proliferation and cell cycle. Functional annotation and GSEA identified immune system-related pathways, cytokine and integrin signalling as important determinants of dormancy throughout time-points. Candidate genes, including *Vcam-1, Axl1, Tyrobp, S100A6* were verified by qPCR and/or flow cytometry and were shown to be up-regulated in dormant cells. These data confirm the persistence of dormant myeloma cells in bone and demonstrate that they have a unique transcriptome profile. Furthermore, we have identified several novel pathways and candidate genes that control dormancy and may be new targets for therapeutic intervention.

Disclosure: The authors declared no competing interests.

P103

P38 MAPK Regulates the Wnt-Inhibitor Dickkopf-1 in Prostate Cancer Cells

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Prostate cancer (PCa) is the most common cancer in men over the age of 50 with a predilection to metastasise to bone in advanced stages. It has been previously observed that the Wnt-inhibitor Dickkopf-1 (DKK-1) is differentially expressed in bone metastasising cancers, directing the osteolytic or osteoblastic phenotype. P38 MAPK (p38) activity is also dysregulated in patients with advanced stages of prostate cancer and has previously been shown to regulate DKK-1 in multiple myeloma. This *in vitro* study seeks to clarify the regulatory function of DKK-1 by p38 in PCa and how this may impact the phenotype of the induced bone lesions. DKK-1 was minimally expressed in MDA-PCa2b and C4-2B cell lines which are known to induce osteoblastic and mixed lesions *in vivo*, respectively. In contrast, the osteolytic PC3 cells have a high level of DKK-1 expression as measured by qPCR and by ELISA ($p<0.005$). Treatment of the osteoblastic cell line C2C12 with supernatants of PC3 cells led to a significant suppression of Wnt3a inducible osteoblastic markers including alkaline phosphatase (ALP) and osteoprotegerin (OPG). Strikingly, there was a strong rescue effect by neutralising DKK-1 with an antibody (anti-DKK-1) or knocking down DKK-1 expression using siRNA. Anisomycin, an activator of the MAPK signalling pathway, significantly increased ($p<0.01$) the expression of DKK-1, whereas p38 inhibitors LY2228820 and SB202190 significantly decreased DKK-1 ($p<0.0001$) expression in PC3 cells. General knockdown of p38 in PC3 cells using siRNA further confirmed the link between p38 and DKK-1 expression ($p<0.0001$). These results indicate the regulation of DKK-1 by p38 in osteolytic PCa cells and future investigations may strengthen the role of both DKK-1

and p38 activity as novel therapeutic targets in PCa-induced osteolytic bone lesions.
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P104

Anti-Tumour Synergy by Sequential Mevalonate Pathway Inhibition using Atorvastatin and Zoledronic Acid in Bone-Seeking Human Breast Cancer

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Breast cancer is the most frequent malignancy in women and often leads to osteolytic bone metastases. Amino-bisphosphonates are anti-resorptive drugs and currently used as adjuvant therapy for affected patients. Similarly to statins, they inhibit the mevalonate pathway, which is essential for the farnesylation and geranylgeranylation of proteins. Here, we demonstrate that this inhibition by atorvastatin and zoledronic acid evokes synergistic anti-tumour effects in human breast cancer cells. Human triple-negative MDA-MB-231 breast cancer cells were treated with 1 μM atorvastatin and 10 μM zoledronic acid. Whereas individual treatments only elicited weak effects, the combination induced a three-fold increase of caspases 3/7 activation and DNA fragmentation as well as a decrease of cell vitality by 60%. The synergistic effects were underlined by the induction of poly ADP ribose polymerase (PARP) cleavage, the reduced phosphorylation of the survival mediator protein kinase B, and a significant reduction of the anti-apoptotic genes B-cell lymphoma 2 (BCL-2) and Survivin (SVV). Furthermore, the treatments significantly reduced the expression and secretion of interleukins 6, 8 and 11 as well as of Dickkopf-1 representing major players in osteolytic bone metastases. Finally, supplementing the cells with either farnesylpyrophosphate (FPP) or geranylgeranylpyrophosphate (GGPP) upon the combination treatment clearly revealed that only GGPP completely abrogated the synergistic anti-tumour effects. These observations show that geranylgeranylation is indispensable for the survival of MDA-MB-231 cells. As functional small Rho-GTPases are geranylated proteins, we assumed an inactivation by the combination treatment. Surprisingly, a concomitant treatment with a Rho-GTPases activator led to even higher anti-tumour effects. In line, a pull down assay revealed an accumulation of the active GTP-bound forms of the Rho GTPases RhoA and CDC42 pointing to a possible anti-tumour mechanism. Our results indicate the synergistic use of mevalonate pathway inhibitors as a new attractive therapeutic approach for the treatment of breast cancer-induced bone metastases.
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P105

Regulation of Cancer-Induced Osteoclastogenesis by Exogenous and Tumour-Derived Sema3A

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Semaphorin 3A (Sema3A), a secreted member of the Semaphorin family, plays a role in tumour cell migration and osteoblast function. Here, we describe a previously unknown role of exogenous and tumour-derived Sema3A in the regulation of cancer cell induced osteoclastogenesis. First, we confirmed that exogenous Sema3A (100 ng/ml) reduced directed (40%, $p<0.05$) migration of the parental "non-bony" human breast cancer cells MDA-MB-231 (MDA-231-P), whereas stable knockdown by short hairpin RNAs significantly reversed these effects (66% increase in directed migration, $p < 0.001$). Next, we went on to demonstrate that exposure of primary bone tumour (Te85 and Saos-2 osteosarcoma cells) and highly metastatic and bone tropic breast cancer (MDA-231-BT) cell lines to exogenous Sema3A (300ng/ml) significantly inhibited the ability of these cells to enhance osteoclast formation in mouse bone marrow cell cultures (Te85, 51% reduction; Saos-2, 48% reduction; MDA-231-BT, 56% reduction; $p<0.01$). Sema3A (300 ng/ml) was also effective in inhibiting RANKL induced osteoclast formation (38% reduction, $p<0.001$), indicating direct and indirect inhibitory effects on osteoclastogenesis. Bone-tropic MDA-231-BT cells expressed significant level of pro-Sema3A, and stable knockdown of pro-Sema3A expression in these cells (40% reduction of protein level, $p<0.01$) enhanced the ability of these cells to stimulate osteoclast formation (70% increase, $p<0.05$). This result implicates tumour-derived Sema-3A in the regulation of tumour – bone cell paracrine crosstalk. In conclusion, these studies suggest that both exogenous and tumour-derived Sema3A inhibit the ability of primary bone and metastatic cancer cells to enhance osteoclastogenesis in bone metastatic microenvironment. When combined with previous published work, these findings suggest that Sema3A, or novel peptides that mimics its action, may be of value in the treatment of osteolytic bone disease. *In vivo* studies to test the effects of Sema3A on mouse models of osteolysis are currently in progress.
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P106

FDG PET/CT is More Efficient than Bone Scintigraphy in Detecting Bone Metastases in Patients with Newly Diagnosed Advanced Lung Cancer: the POUMOS-Image Study

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Background: Non small cell lung cancers (NSCLC) mainly provide osteolytic bone metastases which present a high risk of complications such as severe bone pain, fractures and medullary compression leading to deep functional impairment. Thus, an exhaustive detection of bone metastases is warranted to prevent skeletal complications. Current guidelines of the French Cancer Agency (INCa) do not recommend FDG-PET/CT performance in advanced lung cancers at diagnosis but there are no data comparing bone scintigraphy and FDG-PET/CT to detect bone metastases.
Methods: POUMOS is a non-intervention prospective cohort of patients suffering from adenocarcinoma lung cancers with a first bone metastatic lesion (stage IV). The cohort has been approved by local ethic comity. We included patients from the cohort who had both at diagnosis bone scintigraphy and FDG-PET/CT. We compared the number of bone metastases identified by both methods and also analysed long bone localisations (femur, humerus and tibia).
Results: Among the 63 patients of the cohort, 39 were included in this analysis. Nearly half of them suffered from bone pain and 13% experimented pathological fractures. Spine was involved in 85% of patients and 38% had lower limbs localisation. We observed a significant correlation between bone scintigraphy and FDG-PET/CT for the total number of metastases ($r=0.73$; $p<0.001$) and the number of spine metastases ($r=0.63$; $p<0.001$). FDG-PET/CT identified significantly more lesions than bone scintigraphy for both spine metastases and total number of metastases ($p<0.001$). In addition, FDG-PET/CT identified significantly more long bone lesions than bone scintigraphy (34 vs 24; $p<0.001$) corresponding to 14 lesions ignored by bone scintigraphy.
Conclusion: FDG-PET/CT performed in patients with newly diagnosed lung cancer is more powerful to identify bone metastases than bone scintigraphy. Furthermore FDG-PET/CT identified more bone localisations with a high risk of fracture that could benefit from a specific preventive strategy.
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P107

Rapid Modification of the Bone Microenvironment *in vivo* Following Short-Term Cabozantinib Administration

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Background: Cabozantinib (CBZ), a tyrosine kinase inhibitor targeted against MET and VEGFR2 has demonstrated positive effects in clinical trials of patients with bone metastasis. We hypothesised that treatment responses are partially mediated by cells of the bone microenvironment and report the first detailed characterisation of the bone microenvironment responses to CBZ treatment *in vivo*.
Methods: Nine-week old female GFP-Ob (BALB/cAnNCrl.Cg-Tg(Col1a1-GFP)Row Foxn1$^{nu/nu}$) and 6-week old female BALB/c nude mice (n=4-5/group) received CBZ (30mg/kg, 200µL) or H_2O as control (200µL) 5x weekly for 10 days (8 administrations in total). Bone structure (µCT), osteoblast and osteoclast activity (TRAP, PINP ELISA) and number/mm of trabecular bone, in addition to growth plate cartilage and bone marrow composition (histomorphometry) was determined.
Results: Bone structure was significantly altered in CBZ treated mice (**GFP-Ob:** Bone volume (BV/TV): CTRL: 13.48±0.54% vs. CBZ: 18.75±1.09%, $p\leq0.01$; Trabecular number (Tb.N): CTRL: 2.65±0.13mm^{-1} vs. CBZ: 3.29±0.15mm^{-1}, $p\leq0.05$; Tb.Thickness (Tb.Th): CTRL: 0.0509±0.0012mm vs. CBZ: 0.0568±0.0009mm, $p\leq0.01$; **BALB/c nude:** Tb.Th.: CTRL: 0.0360±0.0012mm vs. CBZ: 0.0431±0.0011mm, $p\leq0.01$). This was accompanied by a reduction in osteoclast number (**GFP-Ob:** CTRL: 5.98±0.46 vs. CBZ: 4.15±0.30, $p\leq0.05$; **BALB/c nude:** CTRL: 8.29±0.39 vs. CBZ: 6.78±0.47, $p\leq0.05$**).** CBZ caused significant growth plate thickening (**GFP-Ob:** CTRL: 0.14±0.01mm^2 vs. CBZ: 0.22±0.01mm^2, $p\leq0.01$; **BALB/c nude:** CTRL: 0.18±0.02mm^2 vs. CBZ: 0.27±0.02mm^2, $p\leq0.05$). We also detected a CBZ-induced increase in megakaryocyte number (**BALB/c nude:** CTRL: 38.91±2.37 vs. CBZ: 47.30±1.77 $p\leq0.05$) in addition to vascular ectasia, reduced bone marrow cellularity and extravasation of red blood cells into the bone marrow.
Conclusions: Our data suggest that CBZ may mediate therapeutic responses partially by modification of the bone microenvironment. (Covered by UK Home Office license PPL40/3531.)
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P108

Abiraterone Exerts a Direct Anabolic and an Anti-Resorptive Activity on Bone Microenvironment

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Background: Abiraterone acetate (ABI) is associated not only with a significant survival advantage in both

chemotherapy-naive and -treated patients with metastatic castration-resistant prostate cancer (mCRPC), but also with a reduced pain from skeletal metastases, a delay in time to development of Skeletal Related Events (SREs) and in radiological skeletal progression. These bone benefits may be related to a direct effect on prostate cancer cells in bone or to a specific mechanism directed to bone microenvironment. To test this hypothesis we have designed an *in vitro* study aimed to evaluate a potential direct effect of ABI on human primary osteoclasts/osteoblasts (OCL/OBL) cultured with/without steroids.

Methods: Primary OCL were differentiated from human monocyte; primary OBL were obtained from mesenchymal stem cells. OCL differentiation and activity were evaluated by TRAP and Bone Resorption assays; OBL differentiation was analysed by ALP and Alizarin Red assays.

Results: Our results showed that non-cytotoxic doses of ABI (5 μM/10 μM) have a statistically significant inhibitory effect on OCL differentiation ($P<0.001$) and bone resorption activity ($P<0.001$) with or without steroids. Moreover, we found that ABI down-modulated the expression of OCL marker genes: TRAP ($P<0.001$), CATHEPSIN K ($P<0.001$), METALLOPROTEINASE-9 ($P=0.001$). Furthermore ABI strongly promoted OBL differentiation ($P =0.02$) and increased bone matrix deposition ($P=0.014$) in presence/absence of steroids. Finally ABI showed a significant up-regulation of OBL specific genes ALP ($P=0.015$) and OSTEOCALCIN ($P=0.034$). The reduction of CATHEPSIN K levels and the increase of OSTEOCALCIN expression were confirmed by western blot.

Conclusions: Overall, these findings represent the first evidence of a novel biological mechanism of ABI consisting in a direct bone anabolic and an anti-resorptive activity. These data may explain, together with the known antitumoural effect on CRPC cells, the high efficacy of ABI treatment in improving multiple skeletal disease specific clinical endpoints (time to SRE, bone rPFS, pain from bone metastases).

Disclosure: The authors declared no competing interests.

P109

High Aldehyde Dehydrogenase Activity Identifies Tumour-Initiating Cells in Human Bladder Cancer

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Accumulating evidence suggests that cancer stem/progenitor cells (CSCs) are involved in tumour relapse and therapy resistance in urothelial carcinoma. These cells seem less affected by anti-proliferative therapies (e.g. chemotherapy), since they are largely quiescent, have an increased DNA-damage response, reside in difficult-to-reach, protective cancer stem cell-niches and express ABC-transporters, which can efflux drugs from the cells. Identifying the cell(s) of origin in bladder cancer and its distant metastases may permit the development of more effective treatment and preventive therapies. In this study, aldehyde dehydrogenase (ALDH) activity was used to isolate and compare $ALDH^{high}$ and $ALDH^{low}$ subpopulations of human bladder cancer cells. $ALDH^{high}$ cells display strongly elevated clonogenic and migratory potential when compared with the more differentiated $ALDH^{low}$ subpopulation. Moreover, $ALDH^{high}$ cells were capable of self-renewal, whereas the $ALDH^{low}$ population was not, as determined with a sphere assay. In line with these data, the mRNA expression levels of several bladder CSC markers including NANOG and KRT14 were significantly increased in the $ALDH^{high}$ cells. Besides the observed effects *in vitro*, $ALDH^{high}$ cells displayed strongly enhanced tumour take and readily formed skeletal metastases *in vivo* while $ALDH^{low}$ do not. In conclusion, our findings suggest that ALDH-based viable cell sorting can be used to identify and characterise tumour-initiating and bone metastasis-initiating cells in human bladder cancer.

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P110

Genetic Variations, Methylation Patterns and Protein Expression in RANK Contribute to Breast Cancer Cell Behaviour in Bone

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Receptor activator of NFκB (RANK) plays an important role in mammary gland development and breast cancer metastasis. Here, we examined the cancer cell-autonomous role of RANK in regulating breast cancer induced osteolysis. First, we observed that RANK is highly expressed in various bone-tropic (BT) clones of parenteral human MDA-MB-231 (MDA-231-P) and mouse 4T1 (4T1-P) breast cancer cells. Epigenetic analysis revealed that enhanced RANK expression in these cells correlates with increased promoter methylation, and further analysis of genetic variations showed that bone-tropic cells predominantly expressed two altered isoforms of the RANK receptor, namely Δ9- and Δ8,9-RANK. Moreover, both isoforms were found to express the regulatory motif for p38 MAPK (^{559}PVQEET^{564}), but lacked TRAF6-binding motif, which is essential for NFκB activation. Mechanistic studies in MDA-231-BT and 4T1-BT cells showed that RANK ligand stimulated p38 phosphorylation (MDA-231-P, 39% and 4T1-P, 20% $p<0.05$), while it had no effects on the phosphorylation of IKKβ and I■B, degradation of I■B and NFκB DNA binding at concentrations up to 300 ng/ml. Functional studies in breast cancer and bone marrow cells demonstrated that exposure of MDA-231-P and 4T1-P or their bone-tropic clones to RANK ligand (100-300 ng/ml) stimulated cell spreading by 2 folds ($p<0.05$), increased 2D random (MDA-231-P, 37%, $p<0.05$), directed (MDA-231-P, 160% and MDA-231-BT, 189%, $p<0.05$) and chemotactic migration (MDA-231-P, 367%, $p<0.05$), and enhanced the ability of bone-tropic breast cancer cells to stimulate osteoclastogenesis (MDA-231-BT, 196% and 4T1-BT, 93%, $p<0.01$), whereas stable knockdown of RANK was inhibitory ($p<0.05$). Collectively, these findings suggest that genetic

variations in RANK in highly metastatic bone-tropic breast cancer cells contribute to phenotypic modifications that alter the host tissue environment to foster formation of osteolytic bone lesions. Studies to further examine the role of different variants of RANK in the regulation of breast cancer induced osteolysis are currently in progress.
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P111
Osteoblast-Derived Factors Increased Metastatic Potential in Human Prostate Cancer Cells
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TGFβ functions as a double-edged sword in prostate cancer tumourigenesis, in later stages promoting invasion and metastatic potential. One well-known cellular source of TGFβ-in the bone metastatic site is the bone-forming osteoblasts, contributing to disease progression through survival and growth of invading tumour cells. Here we have studied the effects by conditioned media from cultures of primary human osteoblasts on migration and metastatic potential in cells from the human bone metastatic prostate cancer cell line PC-3U. Osteoblast-conditioned media resulted in a morphological effect with an increase of long cellular protrusion of the PC-3U cells, an effect that appeared to be dependent on TGFβ-signalling. Also, migration was increased, an effect that was less prominent in PC-3U cells overexpressing a mutated TβRI receptor preventing TRAF6-dependent TGFβ-signalling. Furthermore, osteoblast-conditioned media increased invasive capacity of the PC-3U cells, but decreased proliferation. The effects of the osteoblast-derived factors on the PC-3U cells were not due to epithelial-mesenchymal transition (EMT) or neuroendocrine differentiation. The 3D Matrigel-on-top culture method was used for further evaluation of cell characteristics. Interestingly, treatment of PC-3U cells with osteoblast-conditioned media increased the formation of filopodia-like protrusions (FLPs), previously suggested to promote survival, migration and metastatic potential. In conclusion, the findings presented here suggests that factors secreted from osteoblasts, including TGFβ, can induce several cellular traits involved in metastatic potential of tumour cells, further strengthening the role by bone cells as inducers of metastatic tumour cell behaviour.
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P112
Prostate Cancer Cell-Derived Exosomes Impair Osteoclast Formation and Differentiation
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Skeletal metastatic disease is a deleterious consequence of tumourigenic growth and subsequent dissemination of tumour cells from numerous primary sites, such as prostate, lung and breast. Skeletal metastases are still incurable, resulting in dramatically decreased survival and development of clinical complications such as increased fracture risk, skeletal pain and anaemia for cancer patients with metastatic disease. During the last decade, tumour cell-derived microvesicles have been identified and suggested to be involved in cancer disease progression. Whether cancer exosomes are involved in tumour and bone cell interactions in the metastatic site is still, however, an unexplored field. Here we show that exosomes isolated from prostate cancer cells both decrease proliferation of monocytic osteoclast precursors and clearly impair fusion of osteoclast progenitor cells to mature, multinucleated osteoclasts. Furthermore, the presence of tumour cell-derived exosomes also clearly decreased the expression of established markers for osteoclast fusion and differentiation, including DC-STAMP, TRAP, cathepsin K, and MMP-9. This is, to the best of our knowledge, the first report showing direct effects by tumour cell-derived microvesicles, exosomes, on osteoclast formation and differentiation. Our findings suggest that exosomes released from tumour cells in the tumour-bone interface are involved in the pathological regulation of bone cell formation in the metastatic site. This further strengthens the role of tumour cell-derived microvesicles in prostate cancer progression and disease aggressiveness.
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P113
Vitamin D Reduces Metastatic Prostate Cancer Cell Growth Directly and Indirectly via Stimulation of Osteoblast Differentiation
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Metastases to bone are the incurable final outcomes of cancer, reducing both length and quality of life in an aggressive way. Despite discovery of many factors involved, no cure has been found yet. Bone metastatic cancer cells affect the bone by de-arranging bone metabolism in a way that stimulates cancer cell growth. Compounds that restore bone integrity in bone metastases are promising therapies. One important candidate is vitamin D (1,25-OH_2D_3), known to both stimulate osteoblast differentiation and inhibit cancer cell growth. Our aim is to study the therapeutic possibilities of vitamin D in the crosstalk of osteoblasts and bone metastatic cancer cells and to understand underlying mechanisms. We developed a human co-culture model of differentiating osteoblasts (SV-HFO) and GFP-labeled bone metastatic prostate cancer cells (PC-3). Cells were grown in direct contact co-cultures or mono-cultures for three weeks. Osteoblast growth, differentiation (alkaline phosphatase (ALP) activity) and mineralisation as well as cancer cell growth were monitored in the continuous presence or absence of vitamin D. PC-3 cells significantly ($P<0.00006$) reduced ALP activity in osteoblasts by

74% (32,2 to 8,4 mU/cm^2) starting in the first week and persisting throughout the 3 weeks of co-culture. Mineralisation was almost completely abolished by PC-3 cells. Treatment with 10^{-7} M vitamin D limited the decrease in ALP activity to 20% (32,2 to 25,8 mU/cm^2) and fully restored mineralization. Vitamin D decreased the amount of PC-3 cells by 90% already in the second week of co-culture. In contrast, in the absence of osteoblasts this was only 23% after 2 weeks. These are the first observations that vitamin D may act as a therapeutic agent in bone metastases with a dual function: both directly on cancer cells and via restoring osteoblast activity, and thereby restoring skeletal integrity, quality of life and survival.

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P114

RANK/RANKL/OPG as Predictive Factors of Bone Response in Patients with Bone Metastases from Solid Tumours

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Bone metastases are a frequent event in patients with solid tumours. Although great advances have been made in the treatment of these patients, the identification of new, accurate indicators of bone response would greatly facilitate the clinical management of the disease. The RANK/RANKL/OPG pathway is significantly involved in bone metastasis formation. The main aim of our study was to evaluate the role of circulating RANK, RANKL and OPG levels in predicting bone response. Marker accuracy was also compared with that of the conventional tumour marker NTX. We performed a prospective study on 49 patients with bone metastases from breast, lung and prostate cancer undergoing treatment with ZA. Patients were monitored for one year with blood tests, clinical evaluation, and instrumental exams according to MD Anderson response evaluation criteria and PERCIST. Circulating RANK/RANKL/OPG transcripts and NTX levels were evaluated by Real Time PCR and immune enzymatic assay, respectively. Baseline RANKL levels differed significantly between responders and non responders, whereas no differences in NTX levels were observed in either group. ROC curve evaluation for all markers revealed that RANKL was the most accurate, with an AUC of 0.74 (95%CI 0.54-0.93). RANKL, target of the novel monoclonal antibody Denosumab, was the most accurate predictor of bone response in our series of patients with bone metastases. Its use could potentially improve clinical practice as current bone response evaluation is still somewhat problematic.

Disclosure: The authors declared no competing interests.

P115

Establishment of Patient-Derived Prostate Cancer Models for Drug Development and Personalised Medicine - Fibroblast Growth Factor Receptor (FGFR) Inhibitors as Investigational Drugs

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Background: FGF-signalling pathway seems to have an important role in the progression of metastatic prostate cancer, and FGFR inhibitors have provided interesting preliminary results in preclinical studies. However, many of the currently used preclinical tumour models lack typical microenvironment and characteristics of human disease. The aim of this study was to establish patient-derived prostate cancer models and study FGFR inhibitors as potential investigational drugs.

Methods: Clinical prostate tumour specimens were collected from robotic-assisted laparoscopic radical prostatectomy operations in Turku University Hospital (Turku, Finland). Patient-derived tissues of Gleason grade 7-9 were cut into 1-2 mm^3 pieces and cultured *in vitro* for 6 days with and without androgen supplementation. FGFR inhibitors Dovitinib, AZD4547 and BDJ398 were administered into the tissue culture medium. Viability and differentiation of cultured tissues were examined immunohistochemically by the expression of Ki-67, caspase-3, androgen receptor (AR), PSA and FGF8. Patient-derived xenograft (PDX) *in vivo* models were developed by implanting tissue pieces either subcutaneously or subrenally, or by digesting and then inoculating intratibially into the bone marrow cavity of nude mice.

Results: Tumour tissue maintained its viability in tissue culture and responded to testosterone exposure. Glandular epithelium degenerated when tissue grafts were grown without testosterone, as expected. FGFR-inhibitors decreased proliferation and increased apoptosis of epithelial cells in tissue culture model without having any effect on the expression of AR, PSA or FGF8. In the animal studies, tumour tissue was maintained or even expanded in all used models. Tumour-take was 25% grown in 3 passages.

Conclusion: FGFR inhibitors demonstrated anti-proliferative effects on patient-derived models *in vitro*. Challenging PDX prostate cancer *in vivo* models were successfully established utilising various tumour microenvironments. These models provide a personalised medicine tool that could be used to test the individual prostate cancer patients' responses to therapy in the future.

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P116

Characterisation of the Tumour Environment in Osteosarcoma: M1-Polarised Macrophages and Osteoprotegerin are Two Predictive Factors of the Metastastic Process

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Background: Osteosarcoma is a rare primary malignant bone tumour, with poor prognosis, occurring mainly in adolescents. The bone invasion by a tumour cell affects the balance between bone resorption and formation, releasing factors initially trapped in the bone matrix and which in turn facilitate the tumour development. The main objectives of this work were to characterise the tumour bone microenvironment (or "niche") of localised osteosarcoma or metastatic form of osteosarcoma by histopathological assessment correlated with the clinical annotations.
Methods: Fifty patients suffering from osteosarcoma were enrolled in this study (Nantes University Hospital, France). Analyses were carried out on the pre-operatory biopsies and two groups were defined: localised osteosarcoma (M-) and metastatic osteosarcoma with lung metastases (M+). The tumour niche was characterised by immunohistochemistry approaches: immune cells (macrophages with M1 and M2 polarised macrophages, lymphocytes, masts cells), vascularisation compartment (endothelial cells, smooth muscle cells, pericytes), cytokines/receptors (OPG and RANKL) and mitotic index.
Results: The present cohort included 22 non-metastatic osteosarcomas (M-), and 28 metastatic osteosarcomas (M+). A significant higher infiltration of M1-polarised macrophages was detectable in M- tumours compared with M+ tumours (INOS staining, p=0.001). In addition, M+ tumours exhibited a significantly higher vascular density (CD146 staining, p=0.01) compared with the M- group. OPG immunostaining was significant increased in M- osteosarcomas compared to M+ tumours (p=0.02). In multivariate analyses, INOS and OPG were predictive factors of metastasis (p=0.02 and p=0.03, respectively).
Conclusion: It is an original study, with clinical annotations, comparing tumour bone microenvironment of osteosarcomas with or without metastasis. M1-polarised macrophages and OPG, have predictive factor of the metastastic process. A difference between metastatic or primary osteosarcoma microenvironment was demonstrated leading to propose predictive factors of the metastatic process.
Disclosure: The authors declared no competing interests. This work was supported by the Ligue Nationale Contre le Cancer (Equipe LIGUE 2012) and by INSERM.

P117

Interleukin-34 and Pathogenesis of Osteosarcoma: Involvement in the Tumour Progression and the Metastatic Process by Modulating Macrophage Infiltration and Neovascularisation

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Background: Osteosarcoma is the most common form of primary malignant bone tumour with a high propensity for inducing lung metastases. The tumour microenvironment (cytokines, immune cells, vessels, etc) plays key a role in the tumour growth and in the metastatic process. Interleukin-34 (IL-34) is a cytokine recently discovered by its ability to bind to the M-CSF receptor, to induce the differentiation of monocytes into immunosuppressive M2 and to promote osteoclastogenesis. The aim of the present work was to study the involvement of IL-34 in the pathogenesis of osteosarcoma.
Methods: The expression (qRT-PCR, immunohistochemistry) of IL-34 was assessed in a cohort of human samples. The *in vivo* effects of IL-34 were assessed on tissue vasculature and macrophage infiltration in a murine preclinical model based on a paratibial inoculation of human osteosarcoma cells overexpressing or not IL-34. *In vitro* investigations using endothelial cell precursors and mature HUVEC cells were performed to analyse the involvement of IL-34 in angiogenesis and myeloid cell adhesion.
Results: Our results demonstrated that IL-34 was expressed by human osteosarcoma cells and was regulated by TNF-α, IL-1β. IL-34 overexpression was associated *in vivo* with the tumour progression (tumour growth, lung metastases), an increase of neo-angiogenesis and recruitment of M2-Tumour Associated Macrophages into the tumour mass. *In vitro* investigations showed that IL-34 stimulated endothelial cell proliferation, vascular cord formation and increased monocyte/CD34$^+$ cell adhesion to activated HUVEC under physiological shear stress conditions.
Discussion: IL-34, like M-CSF, plays a part in the inflammatory process by the control of macrophage survival, migration and polarisation. IL-34 may maintain the cancer inflammatory process by facilitating the extravasation of mononuclear phagocytes and may orientate the polarisation of these cells toward an M2 phenotype. IL-34 is a new cytokine clearly involved in the pathogenesis of osteosarcoma and its targeting represents a promising therapeutic approach in oncology.
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P118

Osteoblasts Promote Castration-Resistant Prostate Cancer Growth in Bone by Altering the Intratumoural Steroidogenic Pattern

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Background: The skeleton is the preferred site for prostate cancer (PC) metastasis leading to incurable castration-resistant disease. The increased expression of genes encoding steroidogenic enzymes found in bone metastatic tissue from patients, suggests that up-regulated intracrine steroidogenesis might contribute to tumour growth at the metastatic site. Because of the overall sclerotic phenotype, we hypothesize that osteoblasts regulate the intratumoural steroidogenesis of castration resistant prostate cancer (CRPC) in bone.
Methods: To determine the role of osteoblasts in intratumoural steroidogenesis of CRPC, we accessed the effect of osteoblast conditioned medium (OCM) on the expression of steroidogenic enzymes in androgen-dependent LNCaP, osteogenic LNCaP-19 and osteolytic PC-3 cells using qPCR. Immunohistochemistry was used to detect changes on the protein level in tissue specimens from intratibial, subcutaneous and orthotopic LNCaP-19 xenograft tumours. Serum levels of OPG and RANKL was measured by ELISA.
Results: Osteoblasts induce and alter the steroidogenic transcription program in CRPC cells closely mimicking the gene expression pattern described in bone metastatic tissue from patients. Osteoblast-stimulated LNCaP-19 cells displayed an increased expression of genes encoding for steroidogenic enzymes (*CYP11A1, HSD3B1*, and *AKR1C3*), oestrogen signalling-related genes (*CYP19A1*, and *ER*β), and genes for DHT-inactivating enzymes (*UGT2B7*, *UGT2B15* and *UGT2B17*). The altered steroid enzymatic pattern was bone specific and verified exclusively in tissue specimens from intratibial LNCaP-19 xenograft tumours. Additionally, serum levels of bone-produced OPG and RANKL reflected an increased oestrogen response *in vivo*.
Conclusions: Our results demonstrate that osteoblasts are important mediators of the intratumoural steroidogenesis of CRPC and for castration-resistant growth in bone and therefore need to be taken into consideration in the development of new therapeutic approaches.
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P119

The Activity of Everolimus in a Coculture Model of Breast Cancer and Bone Cells

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Metastatic bone disease has a major impact on both morbidity and mortality of breast cancer patients. Osteolytic lesions are the most common form of bone metastases from breast cancer and are characterised by a significant bone disruption due to a marked increase in osteoclasts number and activity. Alterations in mTOR signalling are involved both in cancer progression and in osteoclasts differentiation. The aim of this study is to highlight the role of mTOR inhibitor everolimus on osteoclastogenesis induced by growth factors or cancer cells media and on cancer cells. We developed an in vitro human model of osteoclastogenesis from peripheral blood monocytes. We used the conditioned media of the osteotropic human breast cancer cell line SCP2. Everolimus was tested at an early step of osteoclastogenesis (5-7 days) and later at 10-12 days of differentiation. Osteoclastogenesis was detected by TRAP assay at day 14. Everolimus was shown to inhibit cancer cells survival of 20%; furthermore SCP2 was able to induce osteoclastogenesis in a significantly statistical manner respect to control. The osteoclast number observed were similar to that obtained with growth factors (differential medium: DM). Everolimus significantly decreased osteoclastogenenis in presence of both conditioned (CM) or (DM). Interestingly, the effect of everolimus was higher if administered to cells early. In this case the inhibition of osteoclastogenesis reached almost the 70%. Finally, with this study we aimed to develop an *in vitro* model that reproduce the interactions between breast cancer cells and the bone microenvironment. In particular, we found a different effect on osteoclast differentiation according to the timing of Everolimus treatment. This drug, interestingly, had an effect on both cancer cells and bone cells. Our model may represent a valid platform for preclinical trials of bone targeted drugs and for the study of molecular mechanisms beyond breast cancer interplay with bone cells.
Disclosure: The authors declared no competing interests.

P120

Tumor-Stroma Cross-Talk: Study of the Osteoclastogenic Potential of a Metastatic Breast Cancer Cell Line in a Coculture System

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Bone metastases represent a major challenge in the treatment of breast cancer, but the complex interactions involved have hampered the development of robust *in vitro* models. The aim of this work is to investigate the osteoclastogenic potential of the osteotropic, human breast cancer cell line SCP2, evaluating its modulation by bone marrow-derived mesenchymal stromal cells (MSC) in presence or absence of the EGFR-blocking compound gefitinib. While conditioned medium (CM) from MSC did not induce osteoclastogenesis in human peripheral blood mononuclear cells (PBMC) we showed that CM from SCP2 and from SCP2-MSC cocultures increased the osteoclastogenesis of PBMC, as evidenced both by functional and molecular assays. Furthermore, the effect of CM from SCP2 treated with gefitinib on osteoclast differentiation was reduced. Moreover, although we couldn't demonstrate a straightforward effect of the EGFR pathway in the osteoclastogenic activity of SCP2, either the treatment of SCP2 with gefitinib or coculture with MSC comparably altered the expression of the bone-related marker RANK. In conclusion, we have developed an *in vitro* human model of coculture of cancer cells, MSCs and osteoclasts, reporting the modulation of the osteoclastogenic potential of SCP2, applying it also in the context of advanced breast cancer drug testing.

Disclosure: The authors declared no competing interests.

P121

Validation of *In Vitro* Cell Viability and Apoptosis Assays for Identifying Compounds that affect Human Multiple Myeloma and Plasma Cell Leukaemia

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Multiple myeloma is a clonal plasma cell malignancy that accounts for 10% of all haematological cancers. Complex interactions of the cancer cells with the bone marrow microenvironment, including activation of osteoclasts and suppression of osteoblasts, lead to lytic bone disease. Heterogeneity of the myeloma contributes to the rapid emergence of drug resistance in high-risk disease. Despite of recent therapeutic advances, myeloma is widely considered as an incurable disease. The responses to treatments depend on the patient and the type of myeloma, and it is therefore of importance to know the direct response to treatment of cancer cells. We have optimised *in vitro* cell viability and apoptosis assays for identifying compounds that affect human multiple myeloma and plasma cell leukemia. LP-1, MOLP-8, RPMI-8226 and OPM-2 human multiple myeloma cells and JJN-3 and L-363 human plasma cell leukemia cells were used in the study. The anthracycline antibiotic doxorubicin was tested as a reference compound with the range of 1 nM to 1 µM concentrations. The cells were cultured for 5 days and the effects of doxorubicin were identified by measuring proliferation of the cells at days 1, 3 and 5 using CellTiter-Glo viability assay. Effects on apoptosis were assessed at day 1 by measuring caspase 3/7 activity using Caspase-Glo 3/7 assay. Doxorubicin showed potent inhibition of cell proliferation at 10 nM concentration in RPMI-8226 and MOLP-8 cells and at 100 nM concentration in all other cell lines tested. There were no major differences of treatment responses to apoptosis induction between the cell lines, with the exception that doxorubicin was more potent to MOLP-8 cells than to the other cells. We conclude that this culture system can be used as a screening tool for finding new chemotherapy agents on multiple myeloma and plasma cell leukaemia.

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P123

Prostate Cancer and Bone Microenvironment: Unveiling the Signature for Bone Metastases

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Prostate cancer is the second most common cancer worldwide for males. The available treatments consist in surgical removal coupled with radio-/chemo-therapy. Prostate cancer metastasises mainly in bone, causing significant increase of clinical morbidity and mortality. Bone metastases develop from a small fraction of the cells shredded by the primary tumour into the blood stream. These cells are able to invade the bone and form metastatic lesions. We aim to uncover the molecular traits characterising these cells thus to predict the risk of bone metastases. Using an *in vitro* co-culture system of osteotropic prostate cancer cells and human osteoblasts, we investigated the interaction between cancer cells and bone microenvironment. We used flow cytometry to investigate the changes in cell cycle at various time points during the co-culture (24 h, 48 h, 96 h). Moreover, we applied to *state-of-the-art* single cell technologies to analyse the expression of target genes and resolve the population composition at a single cell level. Upon co-culture, prostate cancer cells showed progressively increasing proliferation. We also observed significant changes in the subpopulation distribution amongst prostate cancer cells, with genes such as ALDH7A1 and cKit, differentially expressed between co-cultured cells and controls. Dissecting the tumour heterogeneity in the early events of bone colonisation and metastases formation will allow the

development of new diagnostic and curative approaches oriented towards personalised therapy.
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P124
BMP Pathway as a Possible Predicted Marker in Colorectal Cancer
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Bone morphogenetic proteins (BMPs) are multi-functional growth factors primarily isolated from bone according to their osteoinductive capacity and exert their function by BMP pathway including BMP receptors and BMP-related Smad molecules. BMP pathway triggers various processes such as cellular migration, proliferation and differentiation, which are involved in physiological as well as in pathological conditions. As members of TGFbeta family they are also included in processes of epithelial- mesenchymal transition which is important in cancer progression. The aim of this study was to investigate the alteration of BMP pathway in colorectal cancer samples of patients who underwent the CRC surgery. This study was approved by Ethical committee of Clinical Hospital Centre and Faculty of Medicine. All samples were obtained with informed consent. The expression of BMP pathway in different stage of colorectal cancer was characterised by imunohistochemistry and western blot using Smad1/5/8 and pSmad1/5/8 antibody. The clinicopathologic data such as age, gender, Dukes' staging and differentiation grade are also analysed. Additionally, the correlation with establish marker of cell proliferation, Ki67 was examined. Our results showed that colorectal cancer occurred most frequently in men (75%) and in older people (more than 60 years; 85%). The most common site of primary carcinoma was sigmoid colon and rectum (75%). According to Dukes' staging, 55% of examined cancers were Dukes'B and 45% Dukes'C. In both stages, we have found the expression of total and active form of Smad1/5/8 which is higher in Dukes'B stage of colorectal cancer samples. We also found the positive correlation between Ki67 and Smad1/5/8 expression. These results suggested that BMP pathway has also important role in the carcinogenesis and alterations of this pathway could contribute to the cancer progression. Therefore, it could be concern that determination of the BMP pathway activity could be additional prognostic marker of colorectal cancer.
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P125
Upregulation of the Mevalonate Pathway by Cholesterol Depletion Abolishes Tolerance to Nitrogen Containing Bisphosphonate Induced Vg9Vd2 T Cell Cytotoxicity in PC-3 Prostate Cancer Cells
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Background: Nitrogen containing bisphosphonates (N-BPs) are class of drugs used to prevent bone mass. On molecular level they act with inhibition of farnesyl pyrophosphate synthase (FPPS), which leads to accumulation of the isopenthenyl pyrophosphate (IPP) and its transformation by subsequent reaction in the cell cytotoxic ApppI.
Gamma delta T cells are subpopulation of cytotoxic T cells, which are able to recognise and be activated by phosphoantigens such as IPP. PC-3 cell line is an aggressive prostate cancer cell line which shows very low susceptibility to N-BP induced gdT cell cytotoxicity.
Methods: PC-3 cell lines were grown in FK-12 media, 5% CO_2 at 37^0 C. Previously isolated and expanded dgT cells were maintained in RPMI-1640, 5% CO_2 at 37^0 C. Cellular concentrations of IPP and ApppI were analysed with HPLC-ESI-MS and related to the amount of protein. Methyl beta cyclodextrin (mßCD) was used as cholesterol depletion agent. The statistical analysis of the data has been done with Graph Pad software.
Results and conclusions: Prolonged, but not pulse treatment of PC-3 cells with 50 µM zoledronic acid caused some Vg9Vd2T cell cytotoxicity. The addition of mβCD increased susceptibility of the PC-3 cells to gdT cell induced cytotoxicity significantly. Protein expression analysis confirmed that the low basal activity of the mevalonate pathway in PC-3 cells, highly elevated by the cholesterol depletion, is the most probable reason for the natural tolerance of PC-3 cells to N-BPs induced gdT cell cytotoxicity.
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P126
Nanomedicine Design for Bone Microenvironment Targeting in Multiple Myeloma
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Multiple myeloma (MM) is an incurable plasma cell dyscrasia that progresses through stages to overt, symptomatic

myeloma that remains a devastating public health problem. The bone marrow (BM) niche is known to contribute to the growth of tumours such as multiple myeloma (MM) in many ways. Designing better methods of bone-specific delivery for direct and indirect treatment, through the modulation of bone cells, may produce a potent, two-pronged anti-cancer strategy that both heals osteolytic lesions and directly inhibits tumour cells. Nanoparticles (NPs) made from PEG–PLGA coupled to alendronate ("bone-targeted") or alone were formulated and loaded with bortezomib or empty. NPs were characterised for physiochemical properties, bone affinity, drug release profile, and anti-myeloma effects on MM1S myeloma cells *in vitro. In vivo,* anti-myeloma effects, were assessed using bioluminescence imaging (BLI) and effects on bone were done using uCT and IHC. The biodegradable NPs had a uniform size distribution (100-200 nm), with a zeta potential of ±5mV. Bone-targeted NPs showed high affinity towards bone mineral and better bone accumulation *in vivo*. Drug release kinetics showed a burst followed by a sustained-release pattern over 60 hrs. Treatment with bone-targeted bortezomib NPs, non-targeted NPs and free drug worked comparably. In another mouse model, pre-treatment with bortezomib-bone-targeted NPs decreased tumour growth, compared to free drug or non-targeted NPs. Treatment with bortezomib in non-cancer mice increased bone formation in long bones, measured by µCT and histology. Approval of these studies was obtained from our Institution. Bone-targeted nanoparticles hold potential for clinical applications in delivering chemotherapies to bone marrow niches, reducing off-target effects, increasing local drug concentrations, and lengthening the therapeutic window. Future research into how bortezomib modifies the local bone marrow milieu to make it less inhabitable for tumour cells should be explored. Bortezomib-bone-targeted NPs may have potential future clinical utility.

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P127 (CABS OC4.2)

Prostate Cancer Cell Secreted Exosomes Stimulate Osteoclast Formation and Activity

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Prostate cancer (PCa) is the most common cancer and the second cause of cancer-related death in males. When patients have (bone) metastases, no curative therapy is available. MicroRNAs (miRNAs) have been reported for their contribution to PCa metastases, however there is still limited understanding about their actual biological involvement. Cancer cells secrete small vesicles (exosomes) that are enriched for miRNAs. miRNAs within these exosomes potentially have a function in cell-signalling. Secreted exosomes can, when taken up at distant locations, potentially change the microenvironment creating a pre-metastatic niche. The aim of the present study was to investigate the influence of secreted PCa-exosomes on osteoclasts. Exosomes from *in vitro* PCa cell cultures (PC-3, MatLyLu and DU145) and urine were isolated by ultracentrifugation. By small RNA sequencing the miRNAs profile was determined in urine-exosomes from PCa patients. Uptake of PKH67-fluorescently-labelled-exosomes was determined by FACS measurement. Peripheral blood mononuclear cells were isolated by ficol paque separation. Osteoclastogenesis of mouse bone marrow was performed in the presence of M-CSF and RANKL, and osteoclast formation was determined by the TRAP-activity assay. Bone resorption as a measure of osteoclast activity was assessed with Coommassie Brilliant Blue staining. We identified two miRNAs that were differentially expressed in urine-exosomes from metastatic prostate cancer patients compared with patients without metastasis. These were highly present in the blood-exosomes of metastatic patients and also in exosomes retrieved form *in vitro* prostate cancer cell lines. Exosomes were almost exclusively (90%) taken up by human monocytes, which are potential precursors for osteoclasts. After PCa exosome stimulation, osteoclasts differentiation was stimulated, and large multinucleated cells with >10 nuclei/osteoclast were formed, compared with controls with 5-10 nuclei ($p<0.005$). Moreover, we found that exosome-stimulated-osteoclasts were slightly more active in bone resorption, compared with normal osteoclasts. In conclusion, we show that PCa exosomes stimulate osteoclast formation and activity.

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P128 (CABS OP4.2)

Targeting Runx2 By Mir-135 and Mir-203 Impairs Breast Cancer Metastasis and Progression of Osteolytic Bone Disease

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Progression of breast cancer to metastatic bone disease is associated with an aberrantly elevated expression of Runx2, which promotes disease progression through transcriptional activation of genes involved in metastasis. Inhibition of Runx2 in metastatic breast cancer cells prevents metastatic bone disease, thus providing a basis for Runx2 as a potential therapeutic target. Since transcription factors are challenging to target for therapeutic intervention, our goal was to evaluate the potential clinical use of Runx2-targeting microRNAs (miRNAs) to reduce tumour growth and bone metastatic burden. Expression analysis of a panel of miRNAs

regulating Runx2 revealed a reciprocal relationship between the abundance of Runx2 protein and two miRNAs, miR-135 and miR-203. These miRNAs are highly expressed in normal breast epithelial cells where Runx2 is not detected, and conversely are absent in metastatic breast cancer cell lines and importantly, in tissue biopsies that express Runx2. Reconstituting metastatic MDA-MB-231-luc cells with miR-135 and miR-203 reduced the abundance of Runx2 and the expression of the metastasis-promoting Runx2 target genes. Additionally, tumour cell viability was decreased and migration suppressed *in vitro*. *In vivo* implantation of MDA-MB-231-luc cells reconstituted with miR-135 or miR-203 into the mammary gland, followed by additional intratumoural administration of the synthetic miRNAs reduced tumour growth and importantly, spontaneous metastasis to bone. Furthermore, intratibial injection of these cells impaired tumour growth in the bone environment, inhibited bone resorption and secondary metastasis to lung. Importantly, reconstitution of Runx2 in MDA-MB-231-*luc* cells delivered with miR-135 and miR-203 reversed the inhibitory effect of the miRNAs on tumour growth and metastasis. We conclude that aberrant expression of Runx2 in aggressive tumour cells is related to the loss of specific Runx2-targeting miRNAs and that a clinically relevant replacement strategy by delivery of synthetic miRNAs is a viable therapeutic approach to target transcription factors for the prevention of metastatic bone disease.
Disclosure: The authors declared no competing interests.

P129 (CABS OP4.1)
miR-25 and miR-21 Regulate Prostate Cancer Invasiveness by Attenuation of Notch-TGF-β Crosstalk and Self-Renewal Markers
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Altered microRNA (miR) expression is associated with tumour formation and progression of various solid cancers. A major challenge in miR profiling of bulk tumours is represented by the heterogeneity of the subpopulations of cells that constitute the organ and tumour tissue. We analysed the expression of miRs in a subpopulation of bone metastasis-initiating stem/progenitor-like cells in human prostate cancer (PCSC) and compared with more differentiated cancer cells. In PC-3M-Pro4Luc2 and C4-2B prostate cancer cell lines and clinical prostate cancer specimens we identified that miR-25 and miR-21 expression in PCSCs was low/absent and steadily increased during their differentiation into cells with a luminal epithelial phenotype. Functional studies revealed that overexpression of miR-25 in prostate cancer cell lines and selected subpopulation of highly metastatic/ tumourigenic cells (ALDH^{high}) strongly affected the invasive cytoskeleton reducing migration *in vitro*, while overexpression of miR-21 reduced the size of ALDH^{high} subpopulation. Additionally, miR-25 overexpression dramatically decreased the expression of Notch1 and Jagged1, critically involved in aetiology of skeletal metastasis, together with other Notch downstream targets in prostate cancer cells, while miR-21 downregulated self-renewal markers. Moreover, we found that miR-25 decreased TGF-β signaling in human prostate cancer cells and that miR-25 overexpression blocks the induction of Jagged1 driven by TGF-β. In line with these observations, we further demonstrate that miR-25 can act as a tumour suppressor in highly metastatic PCSCs by direct functional interaction with the 3'UTR of pro-invasive α_6 and α_v- integrins. Finally, we show here for the first time, that miR-25 can reduce metastasis by blocking the extravasation of human prostate cancer cells *in vivo.* Taken together, our observations suggest that miR-21 and miR-25 are key regulators of invasiveness in human prostate cancer through direct interactions with α_v- and α_6 integrins & Notch1 expression.
Disclosure: The authors declared no competing interests.
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P130 (CABS OP3.4)
Bone Cells Control Myeloma Cell Dormancy and Activation in the Skeleton
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Multiple myeloma predominantly grows in bone, causing extensive destruction. Despite targeted therapies, relapse is common and the disease remains incurable. To develop more effective treatments we need an improved understanding of myeloma cell engraftment, dormancy and reactivation in the skeleton. We hypothesise that myeloma cells engage in an endosteal niche in which they reside in a dormant state, resist chemotherapy and can be reactivated through changes in the local environment, contributing to disease relapse. To address this, we have developed *intravital* imaging to study tumor cell colonization of the endosteal niche and tumour cell dormancy and reactivation. 5TGM1eGFP murine myeloma cells were labelled with the membrane dye DiD. *In vitro*, DiD label is lost through division, distinguishing dormant (DiD^{+ve}/GFP^{+ve}) from proliferating cells (DiD^{Neg}/GFP^{+ve}). Myeloma cells were injected into C57BLKalwRij mice and treated with melphalan (3 times/week, 5mg/kg days 14-28), sRANKL (daily days 4-6) or vehicle. Using *intravital* microscopy, individual dormant DiD^{+ve}/GFP^{+ve} cells were visualised at 7, 14, 21, or 28 days post injection and located in endosteal niches. By day 14, a limited number of myeloma cells were activated to form growing DiD^{Neg}/GFP^{+ve} colonies which were localised distant from bone surfaces. Melphalan treatment reduced tumour burden (<97%), however dormant DiD^{+ve}/GFP^{+ve} tumour cells remained. Following removal of melphalan treatment, tumor burden increased and DiD^{+ve}/GFP^{+ve} cells reduced, indicating that re-activation had occurred. Lastly, sRANKL stimulation of osteoclast activity reduced dormant

(DiD^{+ve}/GFP^{+ve}) cells, suggesting osteoclast driven increased reactivation of dormant tumour cells. Taken together, these data show that dormant tumour cells, which reside in endosteal niches, resist chemotherapy and are available to repopulate the tumour. Importantly, we demonstrate that increased osteoclast remodelling of the endosteal niche reactivates tumour cells in the skeleton. These data provide insights into the fate of dormant cells, mechanisms behind drug resistance and identifies new mechanisms for disease relapse.
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P131 (CABS OP3.1)
The Inhibition of c-MET Reduces Bone Metastases Induced by Renal Cancer Stem Cells
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Renal cancer patients often develop particularly destructive bone metastases. In solid tumours, cancer stem cells (CSCs) directly promote bone metastasis, thus therapeutic strategies to block the interaction between CSCs and bone microenvironment are currently under investigation. Since c-MET mediates the interaction between cancer cells and mesenchymal cells of the bone microenvironment, we hypothesised that targeting c-MET will lead to bone metastases inhibition. Renal CD105+ CSCs isolated from human cancer patients were injected in NOD/SCID mice, previously implanted with a small fragment of human bone. After the injection of CSCs, mice were daily treated or not with a c-MET inhibitor (JNJ) for 90 days, then sacrificed. Importantly renal CSCs colonised human implanted bone but not mice bone, leading to a specie-specificity of those cells to metastasize human bone. We then found that the JNJ treatment inhibited metastatisation at bone implant site. We studied the effect of JNJ on osteoclasts (OCs) and osteoblasts (OBs) of the bone implant by histomorphometry, showing that CSCs induced an activation of OCs corresponding to an increase erosion surface, whereas the OB activity diminished with a reduction of the osteoid thickness. The treatment with JNJ restored the normal activity of OCs and OBs, comparable with the control mice. Then we investigated the effect of JNJ on *in vitro* cultures of human OCs and OBs, to avoid the bone microenvironment interference. JNJ reduced the number of TRAP+ OCs, whereas it did not significantly affect the number of BAP+ OBs. Furthermore, we analysed mice sera by a multianalyte detection system, showing that IL-11 and CCL20 levels are higher in mice untreated with JNJ than in treated ones, suggesting a role of these molecules in the CSC bone metastatic process. Our results highlight the ability of this c-MET inhibitor to abrogate the bone metastastis formation induced by renal CSCs.
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P132 (CABS OP1.2)
Contribution of Osteocytes to Cancer-Associated Bone Pain via Connexin43-Mediated Communications with Sensory Neurons Under the Acidic Microenvironment in Bone Metastasis
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Bone pain is one of the most prevalent and devastating complications of cancer in bone. The pathophysiology of cancer-associated bone pain (CABP) is poorly understood but likely involves complex interactions among the cancer cells, peripheral sensory nerves and bone cells. Recent studies reported that the calcitonin gene-related peptide-positive (CGRP+) sensory neurons densely innervate mineralised bone, in which numerous osteocytes are present, leading us to hypothesise that osteocytes interact with these CGRP+ sensory neurons to evoke CABP. We tested this hypothesis using an animal model in which inoculation of the JJN3 human multiple myeloma (MM) cells into tibiae induced progressive CABP. We found that JJN3 MM-colonised bone was acidic and that blockade of the acidification by the proton pump inhibitor bafilomycin A1 significantly reduced the CABP. Immunohistochemical examination demonstrated that osteocytes localised in the close proximity of CGRP+ primary afferent sensory neurons in mineralised bone. Co-culture of MLO-A5 osteocytic cells and F11 sensory neuronal cells showed that MLO-A5 cells transferred the permeable living dye calcein to F11 cells by extending dendritic processes to contact the neurites of F11 cells. The general gap junction inhibitor 18β-GA and the selective connexin43 (Cx43) blocker GAP27 and silencing Cx43 in MLO-A5 cells by shRNA all decreased the dye transfer, suggesting that the Cx43 gap junction mediates the osteocyte-sensory neuron communication. Determination of neuronal excitation by Ca^{2+} influx imaging assay showed that the acidic medium excited F11 sensory neuronal cells. Importantly, acid-induced excitation of F11 cells was enhanced in the presence of MLO-A5 osteocytic cells and GAP27 and silencing Cx43 abolished acid-induced F11 excitation in the co-cultures. In conclusion, our results suggest that osteocytes contribute to the pathophysiology of CABP via Cx43-mediated communications with sensory neurons innervating bone. These communications may be a novel therapeutic target in the management of CABP.
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P133 (CABS OP1.1)
Cripto/GRP78 Signalling in Dissemination and Metastasis of Human Osteotropic Prostate Cancer
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Prostate cancer is the most prevalent cancer in men and metastatic spread to bone is detected in up to 80% of the patients with advanced disease. Cell surface oncoproteins are attractive therapeutic targets, readily accessible to antibodies and other membrane impermeable protein/peptide-based anticancer agents. Cripto is a GPI-anchored cell surface/secreted oncoprotein that plays important roles in embryogenesis, stem cell maintenance and tumour progression. GRP78 is a HSP70 family member that binds Cripto at the cell surface. We recently found that Cripto and GRP78 are both highly expressed in human castration resistant prostate cancer (PCa), but not in androgen-dependent tumours. We investigated if Cripto/GRP78 signalling promotes the aggressive, stem cell-like PCa phenotype associated with castration resistance and bone metastasis. To mimic the endosteal metastatic niche, highly metastatic human PC-3M-Pro4luc2 prostate cancer cells were cultured with primary human osteoblasts. We found that the presence of human osteoblasts reduces the proliferation of PC-3M-Pro4luc2 cells and results in induction of the E-Cadherin repressor ZEB1, causing the PCa cells to acquire a more mesenchymal, invasive phenotype as reflected by their reduced E-Cadherin/Vimentin ratio. Co-culture of PC-3M-Pro4luc2 cells with osteoblasts also greatly increased the ALDHhigh/ALDHlow ratio indicating an increase in the size of the metastatic stem/progenitor cell population. This increase in the ALDHhigh subpopulation corresponded to enhanced Cripto and GRP78 expression and stable knockdown of Cripto or GRP78 reduced PC-3M-Pro4luc2 proliferation and clonogenicity, and decreased the size of the metastasis-initiating ALDHhigh subpopulation. Finally, we used zebrafish as a model system for measuring tumour cell dissemination and metastasis and found that Cripto knockdown in PC-3M-Pro4luc2 cells led to a significant reduction in metastatic tumour burden. In conclusion, our findings point to a potential role for Cripto and GRP78 in driving metastatic, therapy-resistant phenotype and suggest that targeting the Cripto/GRP78 pathway may have significant therapeutic potential.

P134 (CABS OP3.2)
Calpain-6 Expression Identifies a Stem Cell Population in Osteosarcoma
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Identification of cancer stem cells in carcinomas has proved to be useful to understand cancer progression and for prognostic purpose. However, the properties of osteosarcoma stem cells remain challenging and controversial, mainly due to the lack of functional markers to study such cells *in vivo*. Previously, we identified calpain-6 as a protective factor involved in chemoresistance process of osteosarcoma. To investigate the mechanisms controlling its expression we characterised 7285 bases of the regulatory sequence in calpain-6 gene. This sequence comprises an active promoter and multiple functional binding sites for embryonic stem cell factors such as Oct4, Nanog and Sox2 as shown by rapid cDNA end amplification and chromatin precipitation. Silencing Oct4, Nanog or Sox2 was sufficient to reduce basal and hypoxia dependent up-regulation of calpain-6 expression and the activity of the regulatory sequence cloned upstream the luciferase gene reporter. This indicates that calpain-6 is controlled by the stem cell transcription factors. To further document a possible relationship between Calpain-6 and a stem cell phenotype, we used GFP as gene reporter, to identify the cells in which the calpain-6 promoter was activated. Culturing osteosarcoma cell lines on non-adherent plastic and in minimal medium allowed obtaining spheroids that were previously shown to be enriched in tumourigenic stem-like cells. Calpain-6 protein was up regulated in spheres obtained from human 143B cells as compared with adherent cultures. Moreover, GFP positive cells sorted from adherent cultures have higher capacities to form spheroids than GFP negative cells. These GFP positive cells also expressed higher RNA levels of the embryonic stem cell markers, c-MYC and ABCB1. Five weeks after injection into the tibia of BALB/c mice, GFP-positive K7M2 cells formed tumours that produced a high luminescent signal as compared with tumours formed from GFP-negative cells that are largely necrotic. In *in vitro* scratch tests, migrating cells were found to express high levels of calpain-6 and GFP-positive cells displayed higher capacities for migration than negative ones, whereas, calpain-6 shRNA reduced these capacities. Finally, intra bone injection of GFP-positive cells resulted in more metastatic lesions in lungs than negative cells indicating that calpain-6 is involved in metastatic process. Altogether, our data show that calpain-6 expression is regulated by transcription factors that control multipotency and renewal of embryonic stem cells. Calpain-6 identifies an osteosarcoma cell population that express stem markers and with higher chemoresistance, migration capacities and tumourigenicity. The reporter system driven by calpain-6 regulatory sequence may therefore represent a powerful tool to further study stem cells in osteosarcoma.
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P135 (CABS OP4.4)
Salinomycin Treatment Inhibits Prostate Cancer Growth In Vitro, In Vivo and in Near-Patient Ex Vivo Models

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Prostate cancer (PCa) is the most common cancer in men, and up to 70-80% of patients with advanced disease present with bone metastases. Current treatment options for metastasised PCa are not curative since hormone, chemo-, and radiation-therapy are relatively ineffective in targeting PCa cells with stem/progenitor-like characteristics (CSCs). Salinomycin, an antibiotic used in poultry, was previously identified in a high through-put screen to target breast CSCs 100x more effectively than paclitaxel. In this study we investigated the anti-tumour effects of salinomycin in human PCa cells *in vitro*, *in vivo* and *ex vivo*. Salinomycin dose-dependently inhibited the proliferation of various human PCa cells (PC3, PC-3M-Pro4, DU145, C4-2B, PC339, PC346C). Interestingly, after establishing docetaxel-resistant cells by serial passaging *in vivo* (PC339-DOC), salinomycin differentially affected docetaxel-resistant cells (vs. parental). Salinomycin induced apoptosis as determined by flow cytometry (Ann/PI) and immunohistochemistry (caspase-3), reduced Notch-signalling (RBPkJ/Luc reporter assay) and inhibited migration of PC-3M-Pro4 cells. When PC-3M-Pro4 cells were FACS-sorted for high aldehyde dehydrogenase (ALDH) enzymatic activity, salinomycin inhibited the clonogenic and sphere-forming capacity of both CSC and non-CSCs equally well. Salinomycin pretreatment of PC-3M-Pro4/mCherry completely blocked extravasation and metastatic colonisation in a zebrafish model with a GFP+ vasculature in which cells were intravascularly injected. Salinomycin pretreatment of PC-3M-Pro4/luc2 cells also reduced the formation of distant metastases in a bone metastasis model of intracardiac injection of cancer cells in nude mice. *Ex vivo*, salinomycin treatment for 7 days (vs. vehicle treated) strongly reduced the number of PCa cells in a novel 'near-patient' model of culturing prostate tumor slices from transurethral resection of prostate cancer tissue (TURP) and bone metastases. In conclusion, salinomycin is effective in inhibiting PCa growth *in vitro*, *in vivo* and in near-patients *ex vivo* models. Therefore, salinomycin may be a promising novel therapeutic approach for the treatment of advanced, bone metastatic PCa.

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P136 (CABS OP1.3)
Endothelin-1, a Gene Regulated by TMPRSS2:ERG Fusion Proteins in Prostate Cancer Bone Metastases

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Bone metastases are frequent and severe complications of prostate cancer (PCa). Recently, the *TMPRSS2:ERG* gene fusion, which results in the aberrant androgen-dependent expression of the ERG transcription factor, has been shown to be the most common gene rearrangement in PCa. This study investigates a potential role of the gene fusion in the development and phenotype of PCa bone metastases. We previously established cell clones from a PCa cell line (PC3c), over-expressing different levels of TMPRSS2:ERG. *In vivo* analysis of bone lesions induced by intra-tibial injections of PC3c-TMPRSS2:ERG clones in mice (ethical approval DR2014-32) showed an increase of osteoblastic phenotype compared with control cells. Furthermore, a transcriptomic study of these clones showed a change of expression in many genes, including *endothelin-1* (*ET-1*). Since ET-1 is known to be involved in osteoblast proliferation and in osteoblastic metastasis formation in PCa, we therefore investigated the transcriptional regulation of *ET-1* by fusion proteins. *In vitro*, we have shown that this gene was overexpressed in PC3c-TMPRSS2:ERG clones, depending on ERG expression levels, and was inhibited by ERG silencing. *In silico* analysis of the promoter of *ET-1* revealed the presence of several potential binding sites of ERG. Chromatin immunoprecipitation experiments demonstrated a direct binding to one of them. Moreover, using a cohort of human carcinoma prostate samples (ethical approval CSTMT-042), we were able to establish a correlation between the expression of *ET-1* and the expression of the fusion gene *TMPRSS2:ERG*, reinforcing the link between *ET-1* and the fusion. Taken together, these results strongly suggest that the *TMPRSS2:ERG* gene fusion contributes to the osteoblastic phenotype of PCa bone metastases and that ET-1 is a crucial target gene regulated by the transcription factor ERG.

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P137 (CABS OP3.3)
EPCR Promotes a Tumourogenic and Metastatic Phenotype in Breast Cancer
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Endothelial protein C receptor (EPCR) is a transmembrane receptor widely expressed in endothelial cells where it exerts cytoprotective and anticoagulant activities. We have shown that it is also expressed in lung tumour cells where it promotes tumour cell survival and increases osseous prometastatic activity. However, to date the contribution of EPCR to tumourogenesis and skeletal metastasis in breast cancer remains ill defined. Lentiviral shRNA-mediated EPCR silenced (shEPCR) cells in the MDA-MB-231 derived 1833 breast cancer cell line showed unaltered growth kinetics in basal or apoptotic-induced *in vitro* conditions. However, EPCR silencing reduced tumour growth in an orthotopic model of mammary fat pad injection. Interestingly, intracardiac inoculation of shEPCR cells led to a substantial reduction in skeletal metastatic burden, assessed by bioluminescence imaging, and osteolytic lesions, evaluated by micro-X-Ray imaging, micro-CT scans and histological analysis. This effect was associated with a decreased skeletal tumour growth observed after intratibial inoculation of shEPCR cells as compared with control cells. Furthermore, after intra-tail injection of the murine breast cancer cell line ANV5, we found a dramatic decrease in lung metastasis in animals injected with shEPCR cells as compared with control mice, despite the similar growth kinetics of the cell lines *in vitro*. *In vivo* transcriptomic analysis identified several relevant signalling pathways differentially altered in shEPCR and control tumours. To explore the clinical relevance of these findings we carried out global expression analysis in a cohort of 286 patients. Patients with high EPCR expression levels had shorter relapse-free survival times as compared with patients with low EPCR expression levels. These data indicate that EPCR confers an *in vivo* protumourogenic and prometastatic phenotype to bone and lung. Monitoring EPCR could represent a clinically relevant factor in breast cancer and a potential therapeutic target.
Disclosure: The authors declared no competing interests.

P138 (CABS OP3.5)
Hif Signalling in Skeletal Progenitors Promotes Breast Cancer Growth and Metastasis Through Systemic Production of CXCL12
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High bone mineral density (BMD) has long been associated with increased risk of breast cancer. Conversely, low bone mass has been correlated with lower risk of breast cancer. Although BMD was initially thought to reflect a cumulative exposure to oestrogens, recent clinical trials demonstrated that high bone mass correlates with elevated breast cancer incidence independently of reproductive correlates, endogenous and exogenous exposure to oestrogen. However, the biological mechanism linking bone mass and the risk of breast cancer is unknown. Our objective was to investigate the role of the osteoblastic lineage in breast cancer, using transgenic mice presenting increased or decreased bone mass (all animal protocols were approved by an animal ethics committee). Here we show that osteoprogenitor cells, targeted by Osterix driven Cre-recombinase, exert a systemic control of breast cancer growth and metastasis. Deletion of the tumour suppressor gene von Hippel Lindau (Vhlh) specifically in mouse osteoprogenitors (Osx/Vhlh$^{fl/fl}$), which results in increased protein level of the Hypoxia-Inducible Factor-1alpha (Hif-1alpha) in these cells, led to increased bone mass, and increased mammary tumour growth and metastasis. Conversely, deletion of Hif-1alpha in osteoprogenitors (Osx/Hif-1alpha$^{fl/fl}$) decreased bone mass, and dampened mammary tumour growth and metastasis. We found that changes in the bone microenvironment are associated with changes in the plasmatic levels of the chemokine C-X-C motif ligand 12 (CXCL12). Pharmacological inhibition of the CXCL12-CXCR4 pathway abolished increased primary tumour growth and dissemination in Osx/Vhlh$^{fl/fl}$ mice. Therefore, skeletal dysfunction alters tumorigenesis beyond the bone microenvironment. Our results provide a mechanistic explanation as for why high bone mass is linked to increased risk of breast cancer, and support the notion that the skeleton is an important organ of the tumour macroenvironment. They also indicate that drugs affecting bone homeostasis may have important consequences in breast cancer.
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P139 (CABS OP4.3)
Radium-223 Dichloride Exhibits Dual Mode-of-Action Inhibiting both Tumour and Tumour-Induced Bone Growth in Two Osteoblastic Prostate Cancer Models
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Radium-223 dichloride, an alpha particle-emitting calcium-mimetic, improves overall survival in prostate cancer patients with symptomatic bone metastases. Here, we define radium-223 mode-of-action and efficacy in two clinically relevant prostate cancer xenograft models. Human LNCaP or patient-derived LuCaP 58 prostate cancer cells were inoculated intratibially and mice were stratified into treatment groups based on lesion grade and/or serum PSA levels. Radium-223 (300 kBq/kg) or vehicle was administered twice at 4-week

intervals. X-rays and serum samples were obtained biweekly. Bone samples were collected for γ-counter measurements, micro-CT, autoradiography and histology. Radium-223 inhibited tumour-induced osteoblastic reaction as indicated by reduced bone volume and surface area in both prostate cancer models. Additionally, radium-223 suppressed metabolic activity in bone as evidenced by decreased osteoblast and osteoclast numbers and reduced PINP levels. Radium-223 treatment also resulted in lower PSA levels as early as two weeks post first dosing, indicating constrained tumour growth. This phenomenon was further supported by reduced tumour area in tibia in both models and an overall increase in necrotic tumour area in the LuCaP 58 model. Moreover, DNA double-strand breaks were increased in cancer cells 24 hours post radium-223 administration in the LuCaP 58 model providing further evidence of anti-tumour effects. Autoradiography confirmed radium-223 deposition in the intratumoural bone matrix in conjunction with osteoblasts. We demonstrate that radium-223 dichloride is successfully incorporated into the intratumoural bone matrix and inhibits tumour growth in both cell line- and patient-derived osteoblastic prostate cancer models. Importantly, given the -particle range of 50-80 µm, potent radiation effects on the immediate tumour microenvironment are expected with minimal or no effects on the more distant bone marrow. Taken together, radium-223 therapy exhibits a dual mode-of-action that impacts on tumour growth and tumour-induced bone reaction, both important players in the destructive vicious cycle of osteoblastic bone metastasis in prostate cancer.

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CELL BIOLOGY: OSTEOBLASTS AND BONE FORMATION

P140 (OP24)

P141 (OP25)

P142 (OP26)

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Homeodomain Protein TGIF is Required for Canonical Wnt Signalling-Induced Bone Formation

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The homeodomain protein TGIF plays crucial roles in tissue homeostasis. TGIF is phosphorylated in numerous cell systems, yet the responsible kinase(s) remains unidentified. In an effort to address this issue, we interrogated Eukaryotic Linear Motif (ELM) database, and found TGIF possesses a potential GSK3β phosphorylation site (T235 and T239). We then showed that GSK3β can directly phosphorylate TGIF, as demonstrated by *in vitro* kinase assays and western blotting using a specific antibody. Functionally, we found mutation of T235/T239 resulted in decreased TGIF turnover, providing an initial hint that phosphorylation by GSK3β might hinder TGIF stability. In fact, suppressing GSK3β activity through either genetic or chemical approaches triggered decreased TGIF polyubiquitination and clearance. Thus, similar to β-catenin, phosphorylation of TGIF by GSK3β leads to its degradation. As GSK3β is a key kinase in Wnt signalling, we investigated whether TGIF played a physiological role in this pathway. Remarkably, expression of TGIF enhanced Wnt-induced gene expression, whereas TGIF deficiency elicited the opposite effects. Mechanistically, TGIF appeared to promote β-catenin accumulation, interfering with the assembly of the β-catenin destruction complex. Furthermore, activation of Wnt signalling induced the expression of TGIF itself, revealing an ability of TGIF to govern a feed-forward loop that sustains Wnt signalling. Given that Wnt signalling is a regulator of osteoblast differentiation and bone formation, we then tested whether TGIF was capable of enhancing this pathway in osteoblasts (OBs) and bone formation *in vitro* and *in vivo*. Expressing TGIF increased OB differentiation in the pre-OB cell lines, ST2 and C3H10T1/2 through Wnt signalling activation, as TGIF depletion was sufficient to blunt Wnt3a-induced osteoblast differentiation in these cells. *In vivo*, TGIF-/- mice display decreased osteoblast differentiation and low bone mass. More importantly, deletion of TGIF prevented the high bone mass phenotype seen in mice harbouring heterozygote deletion of DKK1. This study therefore establishes TGIF as a component of the Wnt signalling machinery that is required for efficient Wnt-induced osteoblast differentiation and bone formation.

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Autophagy in Osteoblasts is Involved In Mineralisation and Bone Homeostasis

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Autophagy is the major catabolic process of eukaryotic cells that degrades and recycles damaged macromolecules

and organelles. During this process, the cytoplasmic material targeted to degradation is delivered to lysosomes upon sequestration within double-membraned vesicles called autophagosomes. Autophagosomes and their contents are cleared upon fusing with late endosomes or lysosomes, and products of these catabolic reactions can then re-enter anabolic and/or bioenergetic metabolisms. Autophagy occurs at low level in all cells to ensure the homeostatic turnover of long-lived proteins and organelles and is upregulated under stressful conditions. In the present work, we analysed the role of autophagy in osteoblasts (OB). We first show that the autophagic process is induced in OB during mineralisation. Then, using knockdown of autophagy-essential genes and OB-specific autophagy-deficient mice, we demonstrate that autophagy deficiency reduces mineralisation capacity. Moreover, our data suggest that autophagic vacuoles are used as vehicles in OB to secrete hydroxyapatite crystals. In addition, autophagy-deficient OB exhibit increased oxidative stress and receptor activator of NF-kB (RANKL) secretion, favouring generation of osteoclasts (OC), the cells specialised in bone resorption. *In vivo*, we observed a 50% reduction in trabecular bone mass in OB-specific autophagy-deficient mice. Taken together, our results show for the first time that autophagy in OB is involved both in the mineralisation process and in bone homeostasis. These findings are of importance for mineralised tissues which extends from corals to vertebrates and uncovers new therapeutics targets for calcified tissue related metabolic pathologies such as osteoporosis.

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Extracellular vesicles (EVs) as shuttles of bioactive molecules: an efficient way of osteoblasts to smartly deliver Receptor Activator of Nuclear factor kappa-B Ligand (RANKL).

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Bone is the site of crowded cell-to-cell crosstalk, and various molecules are exchanged to ensure tissue homeostasis. A new mechanism of intercellular communication is represented by EVs, complex biphospholipidic structures, sized 50-1000 nm. EVs shuttle bioactive molecules, including mRNAs, miRNAs and proteins, to target cells and are involved both in physiological and pathological processes. To investigate the EV-shuttled communication between bone cells, we isolated EV pellets from osteoblast conditioned media (3.03±0.79mg), increasing their yield by 10^{-8}M hrPTH(1-34) (4.05±1.19mg,p=0.0405). By FACS we sorted 16.67±1.93% events showing, by transmission electron microscopy, membrane integrity, and size and structure typical of EVs. EVs shuttled fluorochromes into osteoblasts, monocytes and endothelial cells. 97.1+0.26% EVs contained RNAs transferred to target osteoblasts. 53.95±3.48% EVs were RANKL-positive, which increased up to 63.6±4.20% after PTH treatment (p=0.037). EVs targeted the bone tissue ex-vivo, because murine calvaria, incubated with fluorochrome-loaded EVs, showed fluorochrome integration in bone cells with a vesicular pattern. We injected i.p. 30.000 FACS-sorted RANKL-positive EVs in 5 days-old CD1 pups and observed a fast uptake of EV-shuttled fluorochrome in bone, peaking at 1.5 hours from injection and declining thereafter to a lower plateau within 24 hours. To investigate the in-vivo impact of RANKL-positive EVs on osteoclastogenesis, we injected i.p. 4 days-old $RANKL^{-/-}$ mice with 30,000, 60,000, and 120,000 RANKL-positive EVs/mouse, every other day for 5 times. Tibia sections revealed Tartrate-Resistant Acid Phosphatase (TRAcP) positive cells in treated mice, which were instead totally absent in vehicle-treated $RANKL^{-/-}$ mice. TRAcP-positive cell area steadily increased with increasing EV densities (PBS: ND; 30,000 EVs: 398.92+54.97μm^2; 60,000 EVs: 810.17+169μm^2; 120,000 EVs: 2403.91+932.30μm^2, $p<0.05$), indicating dose-dependent osteoclastogenic potential. Our data demonstrate that EVs are physiologically involved in intercellular communication in bone an contribute to RANKL-induced osteoclastogenesis, representing a potential means of targeted therapeutic delivery.

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The Dual Role of Ebf1 in Osteoblast Differentiation

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Early B cell factor 1 (Ebf1) is a transcription factor that regulates B cell, neuronal and adipocyte differentiation. We and others have shown that Ebf1 is expressed in osteoblasts and that deletion of Ebf1 results in increased bone formation. Conversely, overexpression of Ebf1 in osteoblasts leads to impaired bone formation. The role of Ebf1 during early osteoblast differentiation remains unclear. We aimed to determine which of the effects are autonomous for bone cells and whether Ebf1 could regulate adipocyte and osteoblast lineages. Overexpression of Ebf1 in mesenchymal cell line C3H10T1/2 cells led to enhanced osteoblast differentiation with increased expression of osterix. Conversely, in $Ebf1^{-/-}$ osteoblast cultures differentiation was impaired. To reconcile these data with previous in vivo findings, we hypothesised that Ebf1 could have a dual role in osteoblast differentiation promoting early but inhibiting late stages. To allow for some Ebf1 activity to overcome the early defect in differentiation we used haploinsufficient $Ebf1^{+/-}$ calvarial cells, which showed reduced expression of Osx and ALP. We also identified an Ebf binding site in Osx promoter by ChIP assay suggesting regulation by Ebf1. Interestingly, adipogenesis and adipocyte markers PPARG and aP2 were increased by more than 50 % already on day 7 of $Ebf1^{+/-}$ culture. To confirm these findings *in vivo*, we generated conditional Ebf1 knockout mice,

in which Ebf1 deletion was targeted to early or late OBs by crossing with Osx- or hOC-Cre mouse lines, respectively. Deletion of Ebf1 in early osteoblasts resulted in significantly increased bone volume and trabecular number in the tibia at the age of 12 weeks by mCT analysis, while $Ebf1_{hOC}^{-/-}$ mice did not have a bone phenotype. Our data suggest that Ebf1 promotes early osteoblast differentiation at the crossroads of osteoblasts and adipocytes, possibly via inducing Osx expression. However, Ebf1 inhibits osteoblast function in committed Osx-expressing osteoblasts.

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Normal Bone Formation is Dependent on Both Adequate Serum 25(OH)D Levels and Local Synthesis of 1,25(OH)2D3 in Osteoblasts – Evidence from Mouse Models

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Cells of the osteoblast lineage convert 25-hydroxyvitamin D (25D) to 1,25-dihydroxyvitamin D (1,25D) by virtue of the activity of the CYP27B1 enzyme, which appears to be necessary for the regulation of osteoblastic activity. To further understand the role for local synthesis of 1,25D, we have created two genetically modified mouse models in which Cyp27b1 expression is either ablated or enhanced within mature osteoblasts. Osteoblast-specific Cyp27b1-KO (ObCYP27B1KO) mice were generated with the Osteocalcin-Cre founder line. Osteoblast-specific CYP27B1 transgenic (ObCYP27B1-Tg) mice were generated in which human Cyp27b1 gene overexpression was driven by the 3.6kb human osteocalcin promoter. In both mouse models, no change to serum 1,25D, PTH, calcium, phosphorus or cross-laps were observed. However, six week old ObCYP27B1KO mice demonstrated an 18% reduction on vertebral BV/TV% ($P<0.01$) when compared to $Cyp27b1^{loxP/loxP}$ littermates due to a reduction in trabecular thickness (TbTh) which was associated with a reduction in mineralising surface (MS/BS). No change to osteoclastic bone resorption measures were observed in ObCYP27B1KO mice. Conversely, 7w old ObCYP27B1-Tg transgenic mice exhibited a 14% increase ($P<0.01$) in vertebral BV/TV%, associated with both a 9% increase in TbTh ($P<0.01$) and a trend towards increased MS/BS as well as a 14% increase in serum alkaline phosphatase levels. Although while adult (20w) ObCYP27B1-Tg mice continue to exhibit a 23% increase in vertebral BV/TV%, when raised to be 25D-deplete, the vertebral bone phenotype completely abrogated in ObCYP27B1-Tg mice. Consistent with these findings, MLO-A5 osteoblasts (a mouse cortical bone cell model) exhibited pronounced enhancement of mineralisation in the presence of transient overexpression of the CYP27B1 transgene plus 100nM 25(OH)D. This increase in mineralisation was associated with increased levels of Enpp1 and high levels of Tnap mRNA. Our data strongly suggest that CYP27B1 activity in osteoblasts promotes bone formation and is dependent on adequate supply of 25(OH)D, consistent with our previous studies demonstrating the necessity of adequate serum 25D levels to optimise bone formation.

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SU6656, a Selective Src Kinase Inhibitor, Increases Bone Formation and Bone Mass in Mice

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SU6656 was developed as a selective Src kinase inhibitor, but was later found to affect other signalling pathways. We previously observed that this compound could stimulate osteoblast differentiation *in vitro*. Therefore, we hypothesised that SU6656 could increase bone mass by inhibiting bone resorption through Src inhibition and by stimulating bone formation. To evaluate the effects of this molecule on bone mass, 4-month-old female C57Bl/6J mice received intraperitoneal injections of either 25 mg/kg SU6656 or its vehicle (n=6 per group) every alternate days for 12 weeks. Bone phenotypes were assessed by dexa, microCT, histomorphometry and ELISA assay. Data were analysed by unpaired *t* test. SU6656 treatment did not affect body weight compared with vehicle treatment. In comparison with vehicle-treated mice, SU6656-treated mice exhibited higher bone mineral density (+4.6 %, p=0.013), tibial cortical thickness (+8.5 %, p=0.002), tibial cancellous bone volume (+40.9 %, p=0.002) and trabecular thickness (+17.6 %, p=0.006). SU6656 did not significantly affect serum CTX levels, suggesting that this inhibitor increased bone mass by another mechanism than inhibition of bone resorption. Indeed, SU6656 significantly stimulated mineralising surfaces (1.5-fold, p=0.04 versus vehicle), mineral apposition rate (1.5-fold, p=0.012 versus vehicle) and bone formation rate (2.2-fold, p=0.013 versus vehicle). SU6656 stimulated alkaline phosphatase activity and mineralisation, and potentiated BMP2-induced osteoblast differentiation *in vitro*, while other selective Src inhibitors (PP2 and CGP77675) did not, indicating that SU6656 did not exert its effects through Src inhibition. SU6656 was shown to display a higher selectivity for Yes (another Src family kinase) than for Src. Interestingly, Dovitinib, a Yes inhibitor, also enhanced osteoblast differentiation *in vitro*. In conclusion, our findings indicate that SU6656 increases bone mass in mice by stimulating osteoblast differentiation and thus bone formation, possibly through Yes inhibition.

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Increased Bone Mass and Bone Anabolic Functions in Mature GPR39 Deficient Mice

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Bone is a dynamic structure that undergoes constant bone remodelling. Bone remodelling is regulated by a balance between osteoblastic formation and osteoclastic resorption. Zinc, an essential trace element, is important regulator of bone homeostasis. Recently, a receptor specifically activated by zinc was identified. This receptor named GPR39 belongs to the G Protein Coupled Receptor (GPCR) superfamily. However, a role of GPR39 in regulation of bone metabolism was not explored. In order to elucidate a role for GPR39 in bone metabolism we investigated the bone phenotype of GPR39 deficient mice. These mice had normal body length and weight. However, micro CT analysis of trabecular bone in the femurs of six month old mice revealed a significant 32% increase in trabecular bone fraction compared to wild type littermates. Increased trabecular bone in these mice was a result of higher trabecular number and trabecular thickness. In order to test whether increased bone mass was a result of attenuated resorption by osteoclasts we analysed serum levels of CTX-1 peptide, a marker for bone resorption. CTX-1 levels in GPR39 deficient mice were lower but did not reach statistical significance. On the other hand, serum levels of PINP-1, a marker for bone formation, showed a significant 26% increase in GPR39 deficient mice indicating an increase of bone formation. We compared the mineral secretion of osteoblasts isolated from bone marrow of GPR39 deficient and wild-type mice. The intensity of mineral staining was higher in cultures of GPR39 deficient osteoblasts suggesting an increased function of GPR39 deficient osteoblasts. Our data reveal a novel role for the zinc receptor GPR39 in regulation of bone homeostasis and suggest that its absence leads to excessive bone formation by osteoblasts.

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Design of Nanoparticle-Delivery Systems for Bone Therapies

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Current treatment options for bone-related disorders rely mostly on the systemic administration of therapeutic agents with low solubility/intracellular bioavailability and high pharmacokinetic variability, leading to major off-target side effects. Subsequently, there is an unmet balance between drug delivery and its clinical efficacy. To overcome these issues, nanoparticle-delivery systems might be promising carriers of hydrophobic agents. Therefore, we propose to design a nanoparticle-delivery system for the intracellular controlled-release of small hydrophobic drugs for bone-disorders therapies. To test our system, we selected Dexamethasone as a hydrophobic/lipophilic drug model, known to play a dual regulatory role in bone metabolism/formation depending on dosage and treatment length. Accordingly, a higher (10^{-6}M) or lower (10^{-7} M) concentration of Dexamethasone was loaded into two distinct polymeric-nanoparticles, mPEG-PTMC-copolymer and a FDA-approved-polymer PLGA. The bio-functionality of the developed nanoparticles was further analysed *in vitro*, using MC3T3-E1 pre-osteoblasts and primary bone marrow stromal cells (isolated from wild-type C25BL/6 mice) in contact with Dexamethasone-loaded nanoparticles. Our studies demonstrated that nanoparticles were able to rapidly internalise within 4 hours-of-incubation, modulating the response of osteoblasts to Dexamethasone accordingly with dosage delivered. In fact, the release of 10^{-6}M Dexamethasone by loaded-nanoparticles inhibited significantly osteoblast metabolic activity when compared to non-treated cells ($p<0.001$), while the release of 10^{-7}M Dexamethasone promoted a 2-fold increase in osteoblast activity ($p<0.001$), which was sustained for 14 days. Moreover, the release of 10^{-7}M Dexamethasone also induced osteoblast differentiation, as seen by a trend towards increased alkaline phosphatase activity ($p=0.07$) at day 7-of-incubation and increased osteocalcin levels ($p<0.001$) as well as calcium deposition (matrix mineralization; $p<0.001$) at day 14-of-incubation. In conclusion, we describe for the first time a promising therapeutic strategy for the intracellular delivery of small hydrophobic agents based on polymeric-nanoparticles, demonstrating its *in vivo* therapeutic potential for the delivery of drugs targeting downstream-signalling pathways involved in bone-related disorders.

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The Impact of Visceral Adiposity and Tumour Necrosis Factor-Alpha Expression on the Osteogenic Differentiation Potential of Rat Adipose-Derived Stromal Cells

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Background: We have previously reported that adipose-derived stromal cells (ADSCs) isolated from rat subcutaneous adipose tissue (scADSCs) have osteogenic differentiation potential, whereas ADSCs from perirenal visceral adipose tissue (pvADSCs) do not. However, variations in the perirenal visceral adipose tissue mass (pvATM) of our experimental animals compelled us to investigate whether pvATM affected the differentiation potential of ADSCs, and whether the expression of lineage-specific markers or adipokines in naïve ADSCs could be correlated with differentiation potential in these cells.

Methods: Ethical approval was obtained from the Stellenbosch University Research Ethics Committee (Animal Care and Use). Subcutaneous and perirenal visceral adipose tissue samples were harvested from adult (250 g; ~12 weeks old) male Wistar rats fed on standard laboratory chow, and ADSCs were isolated by collagenase digestion. Based on differences in pvATM weight as a percentage of total body weight, the rats were divided into highVAT (1.261% ± 0.107% SD) and lowVAT (0.207% ± 0.044% SD) groups. Confluent ADSC cultures were treated with either osteoblast differentiation media (OM) or adipocyte differentiation media (AM). Matrix mineralisation and lipid accumulation were quantified with Alizarin Red S staining and Oil Red O staining, respectively. Gene expression levels were measured by semi-quantitative RT-PCR.
Results: Matrix mineralisation occurred in OM-treated cultures of lowVAT pvADSCs, in contrast with the non-osteogenic highVAT pvADSCs. LowVAT ADSCs differentiated into adipocytes after 12 days of AM treatment, compared with 7 days in highVAT ADSCs. Surprisingly, Msx2 and Runx2 expression levels in naïve ADSCs were not indicative of osteogenic or adipogenic potential. Leptin and adiponectin could not be detected, but TNFα expression was detectable in non-osteogenic highVAT pvADSCs.
Conclusions: Increased visceral adiposity and resultant inflammatory cytokine expression in the donor organism may influence the differentiation potential of pvADSCs and possibly scADSCs, and may have implications for cell-based therapies.
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Effects of Macrophage-Stimulating Protein on Osteoblastic Differentiation of C3H10T1/2 Cells

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Macrophage-stimulating protein (MSP) is a serum protein that is activated by members of the coagulation cascade in response to tissue damage. A recent study showed that MSP plays an important role in calcium homeostasis and skeletal mineralisation in zebrafish. However, the precise role of MSP in osteoblast differentiation is not fully understood. In this study, we examined the effect of MSP on osteoblastic differentiation from the mesenchymal lineage C3H10T1/2 cells. Expression of receptor tyrosine kinase RON, which is identified as a receptor for MSP, was significantly increased during osteoblast differentiation, and stimulation by MSP increased the expression of osteogenic markers including alkaline phosphatase (ALP), osteocalcin, and osterix. Furthermore, MSP treatment remarkably enhanced ALP enzyme activity and mineralised nodule formation. Conversely, knockdown of receptor RON attenuated the expression of osteogenic markers by MSP treatment. In MSP-treated cells, the phosphorylation of mitogen-activated protein kinase ERK was significantly increased, and additional treatment with the selective ERK inhibitor PD98059 attenuated the effect of MSP on osteoblast differentiation. Taken together, these results suggest that MSP/RON axis can promote osteoblast differentiation via activation of the ERK signalling pathway.
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Osteogenic Differentiation of Human Mesenchymal Stromal Cell Relies on Autocrine/Paracrine Leptin Activity/Action

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Leptin is an important molecule linking energy to bone metabolism. Although the central and peripheral effects have been extensively studied, the role of locally produced leptin by bone itself has not yet been fully explored. Therefore, we assessed the role of endogenous leptin on osteogenic differentiation of human mesenchymal stromal cells (MSC). Leptin and leptin receptor (LEPR) mRNA were expressed in both MSC-derived osteoblasts and adipocytes but the level of leptin in osteoblasts was 10-fold higher compared to adipocytes. This was confirmed at the protein level, using ELISA. Immunocytochemically, leptin was located throughout the cytoplasm. Next, we assessed whether leptin produced by osteoblasts had an autocrine/paracrine effect on osteoblast differentiation by either blocking the binding to LEPR, using a leptin neutralising antibody (nAb), or by using short hairpin RNA (shRNA) against the LEPR. Compared with untreated osteoblasts, mineralisation was strongly reduced following nAb treatment (-80% at day 14, -40% at day 17 of culture). This was corroborated by inhibition (60-100%) of mineralisation by 3 different LEPR shRNAs. Osteoblast marker genes were unaffected in the first 10 days of culture compared with controls by nAb treatment. Interestingly, while the expression of these genes started to decrease in the control condition after 10 days at the onset of mineralisation, neutralising leptin led to a persistent high expression: collagen I (+300% and +400% at days 14 and 17), alkaline phosphatase (+150% at days 14 and 17) and RUNX2 (+300% at day 17) compared with control. In conclusion, osteoblast maturation and mineralisation require endogenously produced leptin, thereby adding complexity to the role of leptin in bone metabolism. We hypothesise that leptin signalling plays a role in transition to the mineralisation phase and that lack of leptin signalling prevents the necessary downregulation of osteoblast differentiation genes and thereby inhibits the differentiation and delays mineralisation.
Disclosure: The authors declared no competing interests.

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Wnt11 Deficiency and Osteoblast Differentiation

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Wnt-11 signalling uses mainly non-canonical pathways, but it is known to regulate bone formation, possibly through a β-catenin dependent pathway. Here we tested the role of Wnt-11 signalling in bone both *in vivo* and *in vitro*. *Wnt11* knock-out (KO) mice and their wild type (WT) littermates were compared from two to ten months of age. Long bone histology, microcomputer tomography (μCT) and biomechanics were used. Bone marrow derived mesenchymal stem cells were isolated from *Wnt11* knock-out and wild type mice and cultured for 14 days in osteogenic medium. *Wnt11* gene expression was demonstrated with RT-PCR. Procollagen type 1 N-terminal propeptide (P1NP) production and mineralised nodule formation were analysed. RT-PCR showed that *Wnt11* is expressed in mouse osteoblasts at days 3, 7, 9 and 14. Osteoblasts from KO animals proliferated and spread well and attached with numerous focal adhesions on day 14, but the differentiation and mineralisation revealed that *Wnt11* deficiency retarded the osteogenic differentiation. The biomechanical tests and μCT did not show any difference between KO and WT. *Wnt11* deficiency delays mouse osteoblast differentiation and mineralisation. So, Wnt-11 in bone seems not to be indispensable, since bone structure or biomechanical properties were normal in *Wnt11* -deficient mice.

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Characterisation of CD24 Expression in Osteogenic Differentiating Human Stem Cells

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Human mesenchymal stem cells (hMSCs) can differentiate into different cell types, nevertheless the heterogeneity among hMSCs isolates is an important hurdle preventing routinely and robust use and thereby success of hMSCs in regenerative therapies. Gene expression profiling studies in our lab have identified 135 cell surface expressed proteins that were specifically upregulated within 4 days of osteogenic differentiation hMSCs. Here, we characterise one of these cell surface receptors, i.e. CD24, during osteogenic differentiation of hMSCs. Bone marrow-derived hMSCs were differentiated into osteoblasts using dexamethasone and beta-glycerophosphate. The cells were analysed by FACS analysis, biochemical assays, qPCR, KI-67 and EdU incorporation. The percentage of cells expressing CD24 in non-differentiated hMSCs ranged from 2-10% depended on the bone marrow donor and significantly increased 2-3 fold upon osteogenic differentiation within 7 days ($P<0.001$). FACS sort experiments illustrated the existence of 3 different populations within undifferentiated hMSCs based on CD24 expression. 1) CD24 negative cells, 2) CD24 positive cells, and 3) CD24 negative cells that express CD24 upon osteogenic differentiation. Interestingly, CD24 positive cells have significantly reduced alkaline phosphatase expression (1.8 fold) after 7 days of osteogenic differentiation ($P<0.001$) and have strongly reduced mineralisation capacity after 21 days. Moreover, KI-67 staining illustrated a significant lower percentage of proliferating cells among the CD24 positive population than among the CD24 negative population ($P<0.05$) in bone marrow and after 6 days of non-differentiated and osteogenic differentiation. Furthermore, EdU incorporation showed less CD24 positive cells went through S-phase (24.7%) than CD24 negative cells (35%) in the non-differentiated hMSCs ($P<0.05$), however after 7 days of osteogenic differentiation there is no significant difference between the two populations. CD24 positive cells have a reduced proliferation, differentiation, and mineralisation capacity in hMSCs differentiated into osteoblasts. This suggests that cells expressing CD24 are a subset that have a reduced osteogenic differentiation capacity *in vitro* and may have other yet unknown functions. Currently we are further characterising the CD24 positive cells, their role in interaction with other bone marrow cells, and analysing the effects of CD24 knockdown on osteoblast differentiation.

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Increased NF-κb and Reduced Wnt-β-Catenin Signalling Mediate the Altered Osteoblast Differentiation and Function in F508Δ-CFTR Mice

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Cystic fibrosis is an autosomal recessive disorder caused by mutations of the cystic fibrosis transmembrane conductance regulator (CFTR). We previously reported that the prevalent human F508Δ-mutation in CFTR results in decreased bone formation and reduced bone mass in mice. However, the molecular mechanisms by which the F508Δ-CFTR mutation affects bone formation were unknown. In this study, we analysed the impact of the F508Δ-CFTR mutation on the osteoblast phenotype in mice, and determined the mechanisms underlying this phenotype. *Ex vivo* studies showed that the F508Δ-CFTR mutation negatively impacts both the differentiation of bone marrow osteoprogenitor cells into osteoblasts and the function of more mature osteoblasts isolated from F508Δ-CFTR mice compared with wild type mice, demonstrating that the mutation reduces osteoblast differentiation and activity in a cell autonomous manner. Treatment with a CFTR corrector rescued the reduced collagen gene expression in F508Δ-CFTR osteoblasts. Mechanistic analysis

revealed that both NF-κB signalling and transcriptional activity are activated in F508Δ-CFTR osteoblasts. Functional studies showed that the activation of NF-κB transcriptional activity in mutant osteoblasts was associated with increased β-catenin phosphorylation and altered expression of Wnt-β-catenin target genes. Importantly, pharmacological inhibition of NF-κB activity or activation of canonical Wnt signalling improved or corrected the reduced osteoblast differentiation and function in F508Δ-CFTR osteogenic cells. Overall, the results reveal that the F508Δ-CFTR mutation impairs osteoblast differentiation and function, in a cell-autonomous manner, as a result of overactive NF-κB and reduced Wnt-β-catenin signalling. Moreover, this study indicates that targeting these signalling pathways can rescue the osteoblast dysfunctions induced by the F508Δ-CFTR CFTR mutation in cystic fibrosis.

Disclosure: The authors declared no competing interests.

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The Effect of Glucose-Load on Bone Turnover Markers and Osteoblasts Function: an *In Vivo* and *In Vitro* Investigation

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Background: Bone turnover markers (BTMs) are suppressed following a glucose load. Here we examined whether *in vivo* exercise can attenuate the suppressive effect of glucose on BTMs in pre and post-menopausal women. We also examined the direct effect of glucose and insulin on osteoblast apoptosis, viability and alkaline phosphatase (ALP) activity *in vitro*.

Methods: *In vivo*: Eight premenopausal women (age=36.1±2.7years, BMI=25.5±0.8, SEM) and 10 post-menopausal women (age=62.8±2.6 years, BMI=28.3±1.3 $kg{\cdot}m^{-2}$) had an oral glucose tolerance test (OGTT) at rest and 60 min post-exercise (30 min cycling at 70–75% of the pre-determined VO_{2peak}). Blood samples were analysed for serum insulin, glucose, total osteocalcin (tOC), undercarboxylated OC (ucOC), procollagen type I N-terminal propeptide (PINP) and β-isomerised C-terminal telopeptides (β-CTx). *In vitro*: Cultured human osteoblasts (HOBs) were treated for 2 hours with increasing glucose concentrations with or without insulin. HOBs apoptosis, viability and ALP activity were measured.

Results: In both rest and exercise trials, tOC, P1NP and β-CTx were significantly suppressed following OGTT, in both pre and post-menopausal women (8%-14%, $p<0.05$). In post-menopausal women, ucOC was significantly reduced following OGTT, with rest and exercise, compared with baseline despite a significant increase in ucOC post-exercise (~9%, $p<0.05$). In pre-menopausal women, OGTT did not suppress ucOC while exercise significantly increased ucOC (~14, $p=0.039$). Exercise had no significant effect on other BTMs. HOBs apoptosis was higher and viability and ALP activity were lower when cells were treated with 10 and 20 mmol/L D-glucose, compared with 5 mmol/L (all $p<0.05$). Importantly, insulin had a protective effect on all test parameters.

Conclusions: Glucose-load suppresses BTMs in pre and post-menopausal women and acute cycling exercise did not prevent the suppressive effect. BTMs are suppressed due to the higher glucose levels, and not insulin, perhaps due to increase osteoblast apoptosis and a reduction in osteoblast viability.

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Long Non-Coding RNA H19 Positively Stimulates Osteogenic Human Mesenchymal Stem Cells Differentiation and Mineralisation

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The H19 gene was cloned about 20 years ago and was one of the first imprinted genes to be identified. H19 is exclusively expressed from the maternal chromosome (Ariel et al., 2000). The expression of H19 is high during vertebrate embryo development, but it is downregulated in most tissues shortly after birth with the exception of skeletal tissue and cartilage (Lustig et al., 1994). H19 is a long non-coding RNA (lncRNA) that harbours the microRNA-675 in its transcript. Recently it has been shown that microRNA-675 is involved in regulation of type II collagen expression in human articular chondrocytes and may present a potential new target for cartilage repair (Dudek KA et al. 2010). Yet the role of H19 in osteoblasts and mineralisation is incompletely understood. We show that H19 is progressively expressed (about 30-fold increase, $p<0.001$) during osteogenic differentiation of human mesenchymal stromal cells (hMSC). Vitamin D stimulated H19 expression 3-fold ($P<0.001$) in parallel to enhancing mineralisation. Knock-down of H19 RNA by short hairpin RNAs (shRNA) led to 70-95% reduction in alkaline phosphatase mRNA expression and activity and 80-95% inhibition of extracellular matrix mineralisation (P values <0.01 to <0.001). Similar levels of inhibition of the osteoblastic transcription factor RUNX2 and the matrix protein collagen type I expression were observed. H19 expression is paralleled by expression of miR-675 (30-fold increase during differentiation) and knockdown of

H19 suppresses miR-675 expression in osteoblasts. However, yet miR-675 inhibition didn't significantly affect mineralisation. In conclusion, our data show for the first time involvement of the long non-coding RNA H19 in osteoblast biology and its role in regulation of bone formation and extracellular matrix mineralisation. Current data suggest a role of the lncRNA H19 besides the miR675.
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Donor Age-Dependent Influence of Circulating Extracellular Vesicles on Bone Formation *In Vitro*

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Background: Mesenchymal stem cells (MSCs) counteract the decline of physiologic functions but their regenerative power decreases with age. In particular osteogenic differentiation capacity of MSCs has been shown to decline with age thereby contributing to slowed down bone formation and osteoporosis. While much is known about cellular aging of MSCs, little is known about extrinsic factors influencing their functionality. Here we set out to identify circulating factors of the aged systemic environment that affect osteogenesis
Results: While searching for influential factors extracellular vesicles (EVs) were found. Exposition of MSCs to EVs isolated from plasma of human elderly donors failed to induce osteogenesis compared to EVs of young donors. Consequently, the question raised which vesicularly secreted components impact on the differentiation capacity of MSCs. We identified vesicular Galectin-3 as an influential component and could demonstrate that plasma as well as vesicular Galectin-3 levels decline with age in humans. Supportingly overexpression of Galectin-3 in MSCs was shown to boost osteogenic differentiation capacity while reducing its protein expression by siRNA inhibited osteogenesis *in vitro*. Moreover intracellular Galectin-3 levels of MSCs correlated with their osteogenic differentiation potential. Finally, we could show that high vesicular Galectin-3 levels indeed contribute to the pro-osteogenic effect of extracellular vesicles from young individuals.
Conclusion: We could demonstrate that the composition of circulating EVs changes with age and that they deliver factors impacting on the osteogenic differentiation capacity of MSCs. Among other factors vesicular Galectin-3 was shown to be enriched within EVs isolated from young human donors and to enhance osteogenesis. Therefore, reduced vesicular Galectin-3 plasma levels with age might lead to a decreased uptake of Galectin-3 by MSCs and therefore contribute to impaired osteogenesis with age.
Disclosure: The authors declared no competing interests. A patent application describing Galectin-3 as a therapeutic target in deranged bone metabolism has been filed. This work was supported by the European Union (Frailomics and Sybill).

P160

Novel Evidence that the Annexin 2 - CLCX12 Interaction Regulates Osteoblastogenesis in Apolipoprotein A-I Deficient, Osteoporotic Mice

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Background: We have very recently shown that the deficiency of Apolipoprotein A-I (apoA-I), a key-element in HDL biogenesis, results in osteoporosis in mice, under yet unidentified mechanisms. CLCX12 is a chemoattractant cytokine, which is expressed by bone marrow cells of osteoblastic lineage and binds to its receptor CXCR4. Annexin-2 (ANXA2) is an adhesion molecule that directly binds CLCX12 facilitating CXCL12-dependent haematopoietic progenitor cell migration. Herein, we aimed at investigating whether aberrations in ANXA2/CLCX12 interaction are involved in impaired osteoblastic function in apoA-I deficient osteoporotic mice.
Methods: Whole bone marrow cells (WBMCs) were isolated, from the femora of apoA-$I^{-/-}$ (n=6) and wild-type (n=6) C57BL/6 mice and assessed for the expression of the mesenchymal stem cell markers PTPRC/CD45 and SCA1/Ly6, the osteoblast-specific markers Runx2, Osterix, Col1a1, RANKL and the osteoblastic bone marrow niche regulators ANXA2, CLCX12 and its receptor CXCR4, with qRT-PCR.
Results: The expression of the two the mesenchymal stem cell markers examined did not reveal significant differences between the knock-out and the WT group. WBMCs from *ApoA-$I^{-/-}$* mice displayed strongly decreased mRNA levels of the osteoblastic regulators Runx2, Osterix, Col1a1 and RANKL, compared with their WT counterparts. Moreover, the expression of ANXA2 and CLCX12 was significantly reduced, while the expression or the receptor CXCR4 was greatly augmented (possibly via feedback cell reaction-mechanism) in the WBMC of the *ApoA-$I^{-/-}$* compared to the WT mice.
Conclusions: ApoA-I deficiency, results in reduced osteoblastogenic capacity of WBMCs; however, it has no effect on the mesenchymal stem cell pool. This finding implies that apoA-I defficiency affects late stages of MSC progression reducing the entry into the level of bone formation. Additionally, our data provide novel mechanistic evidence that apoA-I and thus HDL may control bone synthesis via the ANXA2-CLCX12 interaction.
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***Morinda Citrifolia* (Noni) Promotes Osteogenic Differentiation**

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There has been a strong interest in searching for natural therapeutics for osteoporosis since natural medicines have fewer side effects and are more suitable for long-term use than synthesised drugs. This study aims to investigate the effects of Noni on osteogenic differentiation. Noni water extract was prepared. C2C12, mouse mesenchymal stem cells (mMSC) and human periodontal ligament cells (hPDL) were used to examine the effect of Noni. In the concentration range that did not show cytotoxicity, Noni significantly increased the expression levels of osteoblast differentiation marker genes, including alkaline phosphatase, Runx2, osterix and osteocalcin, in all of the cell types used in this study. In addition, Noni enhanced matrix mineralisation of mMSC and hPDL cells. Noni increased expression levels of BMP2, Wnt3a and beta-catenin. Furthermore, Noni stimulated TOP-Flash activity which was attenuated by addition of Dkk-1, a Wnt inhibitor. These results suggest that Noni extract promotes osteogenic differentiation of mesenchymal stem cells via enhancing the expression of Wnt ligands and subsequent activation of canonical Wnt/beta-catenin signalling.

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Homocysteine Modulates Mineralisation of Osteoblastic Cells

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Hyperhomocysteinemia is associated with several pathologies such as bone fragility, cardiovascular disease, diabetes, and atherosclerosis. We have recently demonstrated that homocysteine (Hcys) alters collagen cross-linking, perturbs triple-helix formation and regulates expression of genes found in osteoblastic cells, possibly via the inflammation related gene SAA3. Concerning cardiovascular disease, it was demonstrated that Hcys is related to aortic mineralisation in patients with ischaemic heart disease. This finding poses the question whether Hcys influences the deposition of mineral in bone cell cultures as well. For our experiments, we used the pre-osteoblastic cell line MC3T3-E1, which in long-term culture differentiated into mature, mineral depositing osteoblasts. As a second system we cultured MLO-A5 cells. These cells are late osteoblasts, which also deposit mineral, however, already after 10 days. MC3T3-E1 and MLO-A5 cells were cultured up to 5 and up to 3 weeks, respectively. The cultures were treated either with Hcys or ß-glycerophosphate (ßGP) or in combination and mineralisation was determined by Alizarin-red staining. Gene expression was addressed by genome-wide expression analysis (GeneChip, Affymetrix) and expressions of interesting genes were confirmed by RT-qPCR. Long-term cultures of MC3T3-E1 cells revealed that Hcys in combination with ßGP strongly increased the deposition of mineral after 4 and 5 weeks of culture. In MLO-A5 cultures, however, the sole treatment with ßGP stimulated the deposition of mineral, and Hcys had no additional effect. Genome-wide expression analysis and RT-qPCR of Hcys treated cells demonstrated an increase of Phospho1 (phosphatase, orphan 1) and Alpl (alkaline phosphatase), both genes, which are involved in the mineralisation process. Our data suggests that Hcys by up-regulating expression of phosphatases increases the concentration of inorganic phosphate, which accelerates mineralisation of osteoblastic MC3T3-E1 cell cultures. These results also suggest that Hcys can modulate physiological as well as pathological mineralisation processes.

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Cell Fate Modulation of Human Mesenchymal Stromal/Stem Cells by Sulforaphane, a Naturally Occurring Isothiocyanate

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Nutritional drugs have unanticipated anabolic effects on skeletal development and homeostasis. We demonstrated that sulforaphane (SFN), which is abundantly present in cruciferous vegetables like broccoli, has bone anabolic effects in mice via epigenetic mechanisms that mediate active DNA demethylation. We investigated whether SFN has potential utility in skeletal tissue regeneration by controlling differentiation of clinical-grade adipose derived human mesenchymal stromal/stem cells (AMSCs). Our results show that SFN has striking short- and long-term effects on cell growth and differentiation of AMSCs. Administered at an optimal dose of 3 µM, SFN significantly enhances osteoblastic differentiation of as shown by the increased expression of BGLAP2, RUNX2, COLA1A1 and LOX and by the increased mineralisation of the extracellular matrix after 24 days. Apart from osteogenic effects, SFN suppresses adipogenic differentiation of AMSCs. SFN dramatically decreases fat droplet formation and expression of the fat-related PPARY, PLIN1 or CEBPA genes. Mechanistically, SFN induces extensive epigenetic reprogramming of the chromatin within 4 to 10 hours after treatment of the AMSCs. Gene expression profiling using qPCR with a panel of primer pairs for ~400 epigenetic regulators revealed that SFN induces >40 genes involved in chromatin remodelling, including TET3, JHDM1D and KDM6B. Interestingly, long-term effects from SFN treatment on AMSC

differentiation are only achieved by treating the cells within the first 10 hours after induction of differentiation. In contrast, treatment of the cells with SFN without any differentiation media does not shift the cells to a specific cell lineage. These data indicate that SFN extensively enhances chromatin remodelling at the very early stages of differentiation. These epigenetic changes favour osteoblastic differentiation while suppressing adipogenesis. We propose that the natural food compound SFN is an effective agent for modulating cell fate determination of AMSCs, and that SFN may be an effective bone stimulatory in skeletal regenerative therapies.
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Palm Tocotrienol Protects Osteoblasts and Maintain Bone Strength in Rats Exposed to Long-term Glucocorticoid Excess

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Long-term glucocorticoid treatment induces oxidative stress that leads to substantial changes in bone mass, bone structure, mechanical properties and formation of new bone. Free radicals are toxic to osteoblasts and are associated with bone resorption by osteoclasts. Tocotrienol is a type of vitamin E which is an antioxidant and has protective effects against free radical associated diseases. The purpose of this study was to determine the protective effects of palm tocotrienol against glucocorticoid-induced osteoporosis. 40 adult male Sprague-Dawley rats were used in this study. 20 rats were adrenalectomised and replaced with 120 µg/kg/day intramuscular dexamethasone injection. 10 rats were supplemented with palm tocotrienol 60mg/kg/day and another 10 were given apha tocopherol 60 mg/kg/day orally. The control group which consisted of 10 rats was given vehicle palm olein 0.1 ml/kg/day by oral gavage. 10 rats were sham operated and given vehicle palm olein 0.05 ml/kg/day by intramuscular injection and 0.1ml/kg/day orally. The treatments were given for two months before the rats were sacrificed. The right femoral bones were tested for biomechanical strength and the left femoral bones were analysed for cellular parameters of bone histomorphometry. The results showed that long-term glucocorticoid treatment had caused a significant decreased in the osteoblast surface (Ob.S). The Osteoid Volume/Bone Volume (OV/BV) and the Osteoid Surface /Bone Surface (OS/BS) were significantly increased. Supplementation of palm tocotrienol had significantly maintained the Ob.S and the OS/BS equivalent to the sham operated rats. Palm tocotrienol also maintained the biomechanical strength of the bones. This may be due to the antioxidant effect of palm tocotrienol that had protected osteoblasts against the toxic effect of glucocorticoids. This resulted in stronger bone. The results of this study suggested that palm tocotrienol may have protective effects against the adverse effects of excess glucocorticoids and may be used as a supplement for patients on long-term glucocorticoid therapy.
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Abstract withdrawn

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Effectiveness of the Antimicrobial Photodynamic Therapy (Apdt) in the Post-Extraction Socket Healing in Rats Treated with Oncologic Dose of Zoledronate

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Bisphosphonates (BPs) are drugs used in the treatment of bone disease and osteotropics malignant neoplasms. BPs exert inhibitory effect on bone resorption. One of their adverse effects is the bisphosphonate related osteonecrosis of the jaws (BRONJ). The aetiology of the disease is still poorly understood, which makes its prevention and treatment difficult. There are few preventive therapies nowadays. Antimicrobial Photodynamic Therapy (aPDT) presents biological properties that turn it into a promising preventive proposed to avoid the onset and development BRONJ. These study evaluated the action of the aPDT on the osteogenic potential during alveolar repair in rats treated with oncologic dose of zoledronate. Twenty rats were divided in groups: SAL, ZOL, SAL/aPDT and ZOL/aPDT. 0.45mL of solution of 0.9% NaCl (SAL and SAL/aPDT) or 0.45mL of this solution plus 100µg/kg of zoledronate (ZOL and ZOL/aPDT) was administered during 7 weeks, every 2 days, by intraperitoneal injection. After three weeks of treatment, extraction of the lower first molar was performed. At 0, 2 and 4 days post extraction, the aPDT sessions were performed (methylene blue - 100µg/ml; InGaAlP; 660nm; 35mW; 74,2J/cm2; 60s). Twenty 8 days after the surgeries the animals were euthanised. Histological sections of the mandibles were stained with hematoxylin and eosin or prepared for immunohistochemistry for transcription fator Runt-related-2 (RUNX2) and osteocalcin (OCN). The immunostained cells were quantified in the site of tooth extraction. In ZOL group, a small area of newly formed bone was observed in the site of tooth extraction and lot of bone necrosis areas surrounding the dental socket. It presented the highest number of RUNX2-positive cells and the lowest of OCN-positive cells. In SAL, SAL/aPDT and ZOL/aPDT groups, the newly formed bone filled almost all the sites of tooth extractions and they were not found bone necrosis areas, the number of RUNX2-positive cells and OCN-positive cells was similar among these groups. aPDT is presented as an effective preventive therapy in order to improve tissue repair in dental sockets, which is highly compromised by the treatment with oncologic doses of zoledronate.
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Activation of Osmo-Mechanosensitive TRP Channels Facilitate Increase of Ca^{2+}-Mediated RANKL Expression in Mouse Osteoblastic Cells

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Mechanical stress plays an important role in the regulation of bone turnover. However, the intracellular mechanisms of mechanical stress under osteoblast differentiation and proliferation are not well understood. In this study, we investigated the effects of osmo-mechanosensitive transient receptor potential (TRP) channels-induced calcium signalling in primary mouse osteoblasts and MC3T3-E1 cells. Hypotonic stress induced significant increases of RANKL mRNA expression but not OPG. In addition, hypotonic stress-induced increases of intracellular calcium concentration ($[Ca^{2+}]_i$) and RANKL expression persisted in the presence of non-specific Ca^{2+} channel blockers or Ca^{2+}-free bath solution. Furthermore, we examined hypotonic stress-induced effects on agonists and antagonists of osmo-mechanosensitive TRP channels in order to determine the cellular mechanism of hypotonic stress-mediated increases on $[Ca^{2+}]_i$ and RANKL. We found that antagonists of TRPV4 and TRPM3 decreased hypotonic stress-mediated increases on $[Ca^{2+}]_i$ and protein expression levels of RANKL and NFATc1. We also identified that hypotonic stress-induced effects reduced by the genetic suppression of TRPV4 and TRPM3. Taken together, our results indicate that hypotonic stress activates the expression of RANKL and NFATc1 by $[Ca^{2+}]_i$ increases through TRPV4 and TRPM3 in osteoblasts. These effects may be important for the differentiation and proliferation of bone cells on bone remodelling that are mediated via mechanosensitive TRP channels.

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Vancomycin Promotes Osteogenic Differentiation of Mesenchymal Stem Cells in vitro

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Background: Vancomycin is widely used to treat infected bone defects in orthopedic surgery. Several reports highlighted a beneficial outcome if vancomycin-impregnated bone cement was used, but there is little information of direct vancomycin effects on human mesenchymal stem cells (MSCs).
Methods: MSCs were harvested from patients who underwent iliac bone grafting for spine fusion. Cells were cultured in complete medium or osteogenic induction medium. The cytotoxicity assay for vancomycin (range from 0 to 2000 μg/ml) was determined by using WST-1 reagent. After vancomycin (100 μg/ml) treatment, the expression of osteogenic gene was determined by Q-PCR. The alkaline phosphatase (ALP) activity, calcium level, and intensity of Alizarin Red staining of the MSCs were assessed. Protein expression of osteopontin (OPN) and cbfa-1 were detected by western blotting. Phosphorylation of p38 or ERK was evaluated by phospho-kinase array kit.
Results: Vancomycin did not affect cell proliferation until high concentrations (500 ug/ml) for long-termed incubation (14 days). After vancomycin treatment (100 μg/ml), mRNA expression of type I collagen, OPN, Cbfa1 and osteocalcin (OSC) were up-regulated and increased ALP activity and calcium levels were noted. Increased protein expression of OPN and cbfa-1 was shown after vancomycin treatment. Positive Alizarin Red staining through the matrix at the surface layer of the vancomycin treated group was greater than that of the control group. Phosphorylation of p38 and ERK were increased after vancomycin treatment.
Conclusion: Vancomycin dose dependently promotes osteogenic differentiation of MSCs in vitro. Whether MAPK signalling play an important role in these effects remains to be investigated.
Disclosure: The authors declared no competing interests.

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Tumour Necrosis Factor-Alpha Downregulates Uncarboxylated Osteocalcin Secretion via Beta Adrenergic Signalling Pathway in Osteoblasts

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Emerging evidence has suggested important endocrine roles of bone, communicating with other tissues for regulating body energy metabolism. It's been reported that sympathetic nervous system (SNS) activation induces expression of the Esp gene, exclusive to osteoblasts, where Esp gene regulates osteoblastic secretion of the hormone-like substance osteocalcin. An undercarboxylated form of osteocalcin acts as a regulator of insulin in the pancreas. TNF-α is a multifunctional proinflammatory cytokine. Obesity, diabetes and related bone loss is associated with chronic 'inflammation' characterised by abnormal cytokine production such as TNF-α from T cell. We previously reported that TNFα upregulates beta2-adrenergic receptor (β2AR) expression in murine osteoblastic cells and that this modulation is associated with TNFα inhibition of osteoblast differentiation. However the regulatory role of TNF-α in bioactive osteocalcin secretion in osteoblasts has not been investigated. In the present study, we investigated 1) whether TNF-α induces Esp, gene expressions in osteoblast and/or thereby decrease in bioactive osteocalcin and 2) whether down-regulation of TNF-α mediated sympathetic tone with βAR antagonist, propranolol could regulate Esp expression and/or bioactive osteocalcin secretion. C2C12, a murine mesenchymal precursor

cell line was used in this study. Real time PCR and western blot analysis was performed to assess β2AR and Esp gene expressions. For bioactive osteocalcin level measurement, undercarboxylated osteocalcin ELISA assay was conducted. TNFα upregulates β2AR expression in C2C12 cells. Esp expression in C2C12 cells was also upregulated by TNFα treatment, and this increment was attenuated by propranolol. Bioactive osteocalcin level decreased with TNFα treatment and this decrease was mitigated with propranolol treatment. In summary, activation of TNFα signalling in osteoblastic cells downregulates bioactive osteocalcin secretion, partially via βAR signalling pathway. These findings imply that a crosstalk between TNFα and βAR signalling pathways might occur in osteoblasts to modulate body energy metabolism.
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Composite ECM-Alginate Microfibers Produced by Microfludics as Scaffolds with Biomineralisation Potential

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The main purpose of the present study has been the investigation of composite microfibers produced with the combined use of alginate and extracellular matrix (ECM) derived components for bone tissue engineering. We investigated the feasibility to build up novel "bio-inspired materials", in which a hydrogel based scaffold is coupled to an ECM component derived from urinary bladder matrix (UBM) or from collagen (gelatin). The combined use of alginate based microfibers produced by a highly controlled microfluidic procedure, modified through the addition of UBM microflakes or gelatin powder, led to bioadhesive hydrogels, whose architecture, constitutive features and dimensions were investigated with respect to their role on the osteogenic potential of SaOS-2 osteoblastic-like embedded cells. The microfluidic procedure allowed a precise control of the dimensional and morphological characteristics of the microfibers, favourable influencing the viability and function of the embedded cells. Notably, the use of a two inlets micromixing chip resulted in an even distribution of cells and other constituents within the entire volume of the microfibre. We demonstrated that the combined utilisation of alginate (representing the main component of the device) and gelatin solution or UBM particles resulted in a synergistic activity of both materials, positively influencing the viability and 3D colonisation of the embedded cells. Moreover, the bimodal nature of the microfibers provided the ideal environment for the deposition of biomineralised particles as proved by the intense Alizarin Red staining evidenced in relatively short culture time (i. e. 7 days). In this respect, the engineered microfibers represent a smart scaffold offering: (a) the mechanical and material properties of alginate, which can be in turn varied through different gelling conditions such as diverse divalent cations, and (b) the bioactive function given by the presence of gelatin and UBM, improving the viability and osteogenic potential of the embedded cells.
Disclosure: The authors declared no competing interests.

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Effects of Endocannabinoids on Human Periodontal Ligament Stem Cells

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Background: Endocannabinoids, lipid mediators derived from arachidonic acids, are found to be associated with multiple regulatory functions in several tissues. The endocannabinoids anandamide (AEA) and 2-arachidonoylglycerol (2-AG) are produced within bone microenvironment, and EC system has recently been implicated in the regulation of bone metabolism. Human periodontal ligament stem cells (hPdLSCs) are characterised as having multipotent differentiation properties and represents postnatal stem cell sources for regenerative and immunomodulatory therapies. In the present study, the effects of AEA and 2-AG were evaluated on multi-lineage differentiation potentials and related gene expression of hPdLSCs in different designed culture conditions.
Methods: Surface expression of characteristic markers in hPdLSCs was analysed by flow cytometry. Osteogenic and adipogenic differentiation potential of hPdLSCs was evaluated by Alizarin Red and Oil Red O staining, respectively. The proliferation/viability of hPdLCs was measured by 3,4,5-dimethylthiazol-2-yl-2,5-diphenyl tetrazolium bromide (MTT) assay. Gene expression analyses of runt-related transcription factor 2 (RUNX2), osteocalcin (OCN) and collagen type 1 (COL1) by real-time PCR.
Results: hPdLSCs exhibited positive staining to mesenchymal markers CD29, CD90, CD105, CD146 and negative staining to haematopoietic markers CD14, CD34, and CD45 as well as the ability to differentiate in osteoblasts and adipocytes. AEA and 2-AG did not reveal any significant effects on proliferation/viability of hPdLSCs. AEA (10μM) significantly increased COL1 gene expression ($p<0.05$), whereas 2-AG (10 μM) stimulation resulted with significant up-regulation of RUNX2 and COL1 ($p<0.05$). When both endocannabinoids (10 μM, each) stimulated hPdLSCs, significant up-regulation of RUNX2 was observed ($p<0.05$).
Conclusion: AEA and 2-AG appears to have an important modulatory effect on osteogenic differentiation of hPdLSCs. Further studies are needed to elucidate the role of cannabinoid system on hPdLSCs differentiation potential and to evaluate the therapeutic potential of pharmacological modulation of cannabinoid system.

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Does the Enamel Protein Amelotin Promote Collagen-Based Mineralisation?

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Amelotin (AMTN) is a recently discovered protein that is specifically expressed during the maturation stage of dental enamel formation. We have recently shown that AMTN is a potent promoter of calcium phosphate mineralisation *in vitro*, in cell culture, and *in vivo* using transgenic mice that overexpress AMTN in enamel matrix. The objective of this study is to investigate whether AMTN is also able to accelerate mineralisation in collagen based systems. First, we studied the effect of immobilised AMTN on collagen mineralisation *in vitro*. Recombinant human (rh) AMTN was expressed in *E.coli* and affinity purified to near homogeneity. Collagen membranes were immersed in 1 mg/ml rh-AMTN solution in deionized distilled water overnight at RT. Control membranes were immersed in water only overnight. The membranes were then removed from the solution and dried. Each piece was then immersed in SBF mineralisation buffer and incubated at 37°C for 6 days. Scanning electron microscopy of the membranes coated with AMTN revealed numerous calcium phosphate particles associated with the collagen fibres whereas, control membranes with no AMTN coating contained no mineral deposits. Next, we examined the effect of AMTN on mineralization in a collagen-based cell culture system. AMTN expression vector or empty vector (control) was transfected into the mouse osteogenic cell line MC3T3-E1 by electroporation. 24 hours after transfection, culture medium was changed to α MEM containing 4 mM inorganic phosphate and 50 µg/ml ascorbic acid. The mineral nodules were characterised by Alizarin Red staining and transmission electron microscopy. Overexpression of AMTN in mouse calvaria cells also increased the formation of calcium deposits in the culture medium. These findings open up potential applications for the AMTN protein as a regulator of mineralisation in bone and dentin.

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Osteoblast Differentiation of Mesenchymal Stem Cells in Different 3D Culture Models: Issues in the Study of Bone Tumours

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Background: The antitumour potential of new molecules is tested first on tumour cell lines cultured on plastic (2D) and in different animal models. However, less than 10% of the molecules validated by preclinical tests are effective in clinical tests. To reduce this failure rate, an intermediate stage of testing molecules could be achieved in vitro 3D culture systems including the stroma on which cancer cells develop. In order to test new molecules targeting bone tumour cells, we develop 3D scaffold presenting some characteristics of the bone environment.
Methods: Mesenchymal stem cells (MSCs) derived from human bone marrow, were cultured either on a biphasic calcium phosphate ceramic (BCP) or nanofibers of polycaprolactone in osteogenic medium (100 nM dexamethasone, 250 µM ascorbic acid, 10 mM β-glycerophosphate). Cell viability studies, electron microscopy, immunohistochemistry (alkaline phosphatase, type I collagen and bone sialo protein), quantification of mineralisation (Alizarin Red) and collagen (Sirius Red) and studies gene expression (*COL1A1*, *CBFA1*, *ALP*, *SOST*, *BSP*, *BMP2*, *OC*) were performed.
Results: Viability of MSCs was observed on long culture times (30-90 days) and their differentiation to the osteoblast phenotype was confirmed on both 3D culture systems. The abundant presence of type I collagen and the presence of non-collagenous bone proteins were observed, indicating the synthesis of an osteoid extracellular matrix.
Conclusion: These culture systems have not resulted in a lamellar bone tissue *in vitro*, but an osteoid matrix was obtained and used to culture osteosarcoma or Ewing's sarcoma cells.

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Upstream Regulator Analyses of Gene Expression Profiles from Differentiating hMSCs Identifies Hoxa9 and Irf2 as Regulators of Osteogenic Differentiation

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Ageing of bone is associated with decreased bone mineral density (BMD) and is caused by increased bone resorption by osteoclasts that is not sufficiently compensated by

an increase in bone formation by osteoblast. In addition, increased adipose tissue volume is observed in the bone marrow cavity caused by enhanced adipogenesis in aging and osteoporotic people. Since osteoblasts and adipocytes share a common precursor in the bone marrow (Mesenchymal Stromal Cells, MSC), we wanted to assess the early regulatory events that occur upon differentiation of human MSCs into both lineages. To address this question, we have generated gene expression profiles of early osteogenic and adipogenic differentiating human MSCs (11 time points within 4 days) by Illumina microarray analyses. The data were dissected by bioinformatic means (a.o. gene ontology (GO) and upstream regulator analyses) and functional overexpression studies. Unsupervised cluster analyses of our gene expression profiles illustrated that the adipogenic and osteogenic differentiated cells can be separated within 3 hours after the initiation of differentiation and reach a stable phenotype after 2-3 days. GO analyses demonstrated that transcription factor (TF) activity ($p<10^{-9}$) was significantly enriched among the differentially expressed genes within the first 3 hours. This was followed by more lineage specific categories such as extracellular matrix proteins (12 hours, $p<10^{-8}$) in osteogenic and oxidation-reduction (24 hours, $p<10^{-2}$) in adipogenic differentiating hMSC. Analyses of TF activity identified 10 TFs that were (in)activated (z-score <-2 or >2) within the first 3 hours in both lineages. The activity of 4 and 20 TFs changed specifically upon osteoblast or adipocyte differentiation, respectively. These included known TFs involved in hMSC differentiation as well as TFs that have not been described before to be involved in the differentiation of osteoblasts. Overexpression of two TFs, HOXA9 and IRF2, illustrated enhanced mineralization confirmed their role in osteogenic differentiation of human MSCs.
Taken together, we describe at the genetic level the osteogenic and adipogenic MSC differentiation. TFs are among the first proteins that are differentially expressed and (in)activated upon differentiation. We have identified interesting TFs that hold the potential as early regulators of lineage decision.
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P175
Devitalised Extracellular Matrix Produced by Osteoblasts Accelerates Osteogenic Differentiation of Mesenchymal Stromal Cells
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Bone is composed of extracellular matrix (ECM) that physically supports bone cells and actively influences their behaviour and differentiation. In the bone marrow, Mesenchymal Stem Cells (MSCs) are in close contact with the ECM and bone cells. Due to their osteogenic differentiation potential and paracrine activity, MSCs are promising candidates for bone tissue engineering applications. The impact of the ECM on MSC behavior was investigated by using an *in vitro* model of devitalised ECM, to exploit it as a natural biomaterial to enhance the osteogenic potential of MSCs. Human MSCs were differentiated into osteoblasts and devitalised by freeze-thaw cycles and DNAse treatment to produce the ECM. Its proteomic composition was analysed by mass spectrometry. Next, MSCs were seeded on the ECM and monitored for cell adhesion, proliferation, osteogenic differentiation and mineralisation. The MSC-derived ECM was metabolic inactive. 846 proteins were detected: significantly enriched ones (P<0,01) were involved in ECM structure, cell-matrix adhesion, related to mitochondrial activity and calcium binding. The ECM significantly enhances MSC adhesion already 2 hours after seeding (number of focal adhesions/cell area (FA/um^2) on ECM=4,9^{-04} and on plastic=2,6^{-05}; P<0,001). ECM induces a temporal increase of MSC proliferation: the percentage of proliferating cells was about 3 fold higher 24 hours after seeding MSCs on ECM compared with plastic (P<0,001). MSCs on ECM are accelerated in their osteogenic differentiation compared to MSCs on plastic: the peak of Alkaline Phosphatase was detected earlier and 5 fold increased (P<0,01). This results in a significantly enhanced mineralisation (P<0,001): quantification of Alizarin Red Staining shows that MSCs cultured on the ECM for 13 days mineralised, whereas the control did not yet. The devitalised ECM increased adhesion of MSCs and temporally enhanced their proliferation. MSC osteogenic differentiation was accelerated, enhancing the mineralisation. These properties make the devitalised ECM a suitable microenvironment to improve MSC utilisation for bone regeneration. Identification of key molecules responsible for the observed effects will unravel their potential for using in bone tissue repair applications
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Cell Attachment and Proliferation of Bone Marrow-Derived Osteoblast on Zirconia of Various Surface Treatment
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This study was performed to characterise the effects of calcium phosphate coated and hydroxyapatite coated zirconia compared with smooth surfaced zirconia with bone marrow-derived osteoblast culture. Bone marrow-derived osteoblasts were cultured on (1) smooth zirconia, (2) zirconia coated with calcium phosphate (CaP), and (3) zirconia coated with hydroxyapatite (HA). The tetrazolium-based colorimetric assay (MTT test) was used for examining the attachment of cells. Cellular morphology was examined by scanning electron microscopy (SEM) and alkaline phosphatase (ALP) activity was measured to evaluate the cell differentiation rate. X-ray photoelectron spectroscopy (XPS) was employed for the analysis of surface chemistry. The genetic expression of the osteoblasts and dissolution behaviour of the coatings were observed. Analysis of variance (ANOVA) was conducted to assess the significance level of the differences between the groups.
From the MTT assay, there was no significant difference between smooth zirconia and surface coated zirconia ($p>0.05$). From the SEM image, cells on all three groups of

discs were irregularly triangular or elongated in shape with formation of filopodia. From the ALP activity assay, the optical density of osteoblasts on smooth zirconia discs was slightly higher than that of osteoblasts on surface treated zirconia discs ($p>0.05$). Most of the genes related to cell adhesion showed similar expression level between smooth zirconia and surface treated zirconia. The dissolution rate of Ca^{2+} and P^{-} was higher with CaP coating than HA coating. The attachment and growth behaviour of bone-marrow-derived osteoblasts cultured on smooth zirconia and surface coated zirconia showed comparable results. However, the HA coating showed more time-dependent stability compared with the CaP coating.
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P177
Structural Functional State of the Mandibular Condylar Cartilage after Implantation of Manganese Enhanced Hydroxyapatite into the Tibia
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Background: The aim was to investigate of structure of the mandibular condylar cartilage (MCC) after implantation of manganese (Mn) enhanced hydroxyapatite into the tibia.
Methods: The study involved 252 male rats with initial body weight of 135-145 grams. The 1st group comprised intact animals, the 2nd group comprised animals with a plain 2.2 mm defect in the tibia, and the groups 3 through 6 comprised the animals with the same 2.2 mm defects filled with biogenic hydroxylapatite enhanced with 0.1%, 0.25%, and 0.5% share of Mn. Upon expiration of observation terms, the frontal sections of the MCC were hematoxylin-eosin stained and put to light microscopy for measurements of MCC zones (V. Luzin, 2012).
Results: A plain defect in tibia had inhibiting effects on activities of MCC. Peak of alterations was registered by the 60th day when zones of proliferation (ZP) and osteogenesis (ZO) were narrower than controls by 9.55% and 12.38%. In the 3rd group, alterations in comparison with the 2nd group continued manifesting by the 7th and the 15th days. By the 30th day, restoration of MCC seemed to be faster and finally by the 60th day ZP and ZO were wider than those of the 2nd group by 4.17% and 5.28%. Implants with 0.1% of Mn share had nearly the same effect and few significant differences were revealed. With Mn concentration increase, restoration rate of MCC appeared to be higher starting from the 15th day. Maximum value gap between the 3rd and the 5th groups was observed by the 60th day: ZP and ZO were wider than those of the 3rd group by 5.87% and 7.16%. Mn share of 0.5% did not have positive effect on MCC restoration. What is more, from the 60th to the 180th day density of osteoblasts in ZO was lower than that of the 3rd group by 4.23%, 5.01%, and 5.91%. This can be explained as Mn intoxication.
Conclusions: The results obtained show that a plain 2.2 mm defect in the tibia has adverse effects on activities of MCC. Implantation of pure hydroxyapatite produces no visible effects in early observation terms (the 7th and the 15th days) and starting from the 30th day alterations reduce faster. Application of Mn enhanced implants significantly reduces negative effects of bone fracture on MCC. Implants with 0.25% share of Mn proved to be the most effective while implants with 0.5% share of Mn produced signs of Mn intoxication.
Disclosure: The authors declared no competing interests.

P178
The Role of NF-κb Regulator Bcl-3 in Osteoimmune Health and Disease
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Bone erosion and fragility fractures are associated with rheumatoid arthritis and postmenopausal osteoporosis, representing a major unmet clinical problem. In health, the balance between osteoblasts and osteoclasts is a dynamic process under tight regulation. In disease, regulation is uncontrolled resulting in overt bone loss. NF-κB is a master regulator of cellular function and is an essential element in the development and homeostasis of the skeletal system. As such, it is a critical controller of both osteoblast and osteoclast differentiation and function. Bcl-3 is an atypical IκB protein and via its selective interaction with homodimers of NF-κB is a critical negative regulator of cellular function. Work in our laboratory has now established that mice deficient in Bcl-3 have increased bone mass, and that *in vitro* culture of their osteoblast precursors results in enhanced differentiation and function. Here, we investigate the role Bcl-3 plays in osteoblast and osteoclast generation and function. Furthermore, *in vivo* studies will determine whether the loss of Bcl-3 can provide protection from bone loss in a murine model of postmenopausal osteoporosis. These studies will provide preclinical supporting data to validate Bcl-3 as a novel target for the treatment of osteoporosis and other diseases associated with bone pathology.
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P179
Low Adhesive Scaffold Collagen, Inducing Spheroid Formation, Promotes the Osteogenic Differentiation
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Background: Osteoblasts are distinguished by the characteristic morphology and by the deposition of the mineralised matrix. Another characteristic is the synthesis of type I collagen and other specialized bone proteins. Since collagen has biocompatibility with bone, collagen is a poor bone graft material when used alone. Therefore, much attention has been directed to find the biomaterial that induces osteogenic progenitor cells. However, best material still remains to be developed. It is essential to clarify the molecular mechanism

of osteogenesis and understand how it induces. We succeeded in developing low adhesive scaffold type I collagen (LASCol) by enzyme treatment (patent pending). In this study, we report that LASCol markedly facilitates osteogenic differentiation of rat marrow mesenchymal stem cells (rMSCs). Furthermore, we investigated the effects of bone wound healing by implanting LASCol in a defect of rat shinbone.
Methods: We obtained LASCol by the enzyme treatment of pig type I collagen. Culture dish was coated with LASCol or pepsin-treated collagen (AteloCol). MC3T3-E1 cells or rMSCs were cultured with osteogenic basal medium on each coated-dish. We observed cell morphology by using a phase-contrast microscope. We analysed the activity of mineralisation by Alizarin red S reagent to evaluate the osteogenic differentiation. Moreover, we transplanted each collagen graft into 2.5 mm critical-sized defects (CSDs) of SD rat shinbones. After 15 days, bone repair efficiency of CSDs was evaluated by HE stain and micro-CT observation.
Results: MC3T3-E1 cells formed the spheroid morphology by only culturing on the LASCol-coated dish. By adhesion to LASCol, the activity of mineralization of MC3T3-E1 and rMSCs were significantly promoted. We demonstrated that LASCol graft induces bone regeneration of rat shinbones and is more bioabsorbable material than AteloCol.
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New Methods for the Separation and Identification of the Osteogenic Compounds of Nacre

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Currently, osteoporosis has become a worldwide major health concern. To increase the bone mineralisation and prevent osteoporotic fracture, nacre is a promising solution. Nacre, or mother of pearl, is capable to increase the cell osteogenic activity. We have confirmed the osteogenic potential of a particular nacre extract, ESM (Ethanol Soluble Matrix) on osteoarthritis (OA) osteoblasts, which are known to have difficulties to mineralise. But those nacreous osteogenic compounds have not been identified yet. Herein, we developed some new methods to identify the active compounds. We evaluated their mineralisation induction capacity on OA osteoblasts. Therefore, ESM was extracted with ethanol from nacre powder of pearl oyster *Pinctada margaritifera.* We selected molecules with ion-exchange resin to achieve cationic ESM (ESMc) and anionic ESM (ESMa). Both were then tested for 28 days at 200µg/ml on subchondral osteoblasts from OA patients undergoing total knee replacement. Alizarin Red staining was performed to test their capacity of mineralisation and quantified at 405 nm. Size-exclusion and strong ion-exchange HPLC were used to separate ESMc and ESMa and compounds were then identified by mass spectrometry. Alizarin Red assay demonstrated an increase of calcium deposition in OA osteoblasts in presence of ESMc. But in presence of ESMa no mineralisation is observed. HPLC and mass spectrometry showed that ESMc and ESMa were composed differently. In conclusion, we acquired 2 totally different fractions from ESM, ESMc and ESMa. ESMa did not exert any mineralisation induction capacity on OA osteoblasts. The osteogenic compounds of ESM are cationic. This strategy associating ion-exchange resin, OA osteoblasts, HPLC and mass spectrometry could be effective for the identification of nacreous osteogenic molecules. This improvement will advance the treatment of osteoporosis with nacre extract.
Disclosure: The authors declared no competing interests.

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IL-17 Augments Osteoblast Differentiation and Bone Regeneration

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The interleukin-17 (IL-17) is a pro-inflammatory cytokine that are involved in the immune response of tissues but also play a role in bone metabolism. It was previously shown that IL-17 accelerated osteogenic differentiation of human mesenchymal stem cells while inhibited osteoblast differentiation and bone regeneration in rats. To delineate the apparent discrepancy, we evaluated the effect of IL-17 on osteogenesis in mice both *in vitro* and *in vivo*. When mouse calvarial osteoblast precursor cells were cultured for in osteogenic medium in the presence of IL-17, the alkaline phosphatase activity and the expression of alkaline phosphatase, osteocalcin, and osterix were significantly enhanced. Moreover, IL-17 significantly accelerated bone regeneration in mouse calvarial defect model. Interestingly, the expression profile of mouse osteoblast IL-17 receptor genes was similar to that of human mesenchymal stem cells, while contradicted with that of rat cells. Our results suggest a species or cell type-specific role for IL-17 in bone formation.
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Viability and Profileration of Human Adipose-Derived Stem Cells on Poly(ε-Caprolactone) Films for Bone Tissue Engineering Applications

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Bone tissue engineering represents a challenge in regenerative medicine. Many type of materials have been studied as

scaffolds in combination with mesenchymal stem cells, in order to develop viable substitutes able to restore and maintain the function of human bone tissues in skeletal defects. In this work, we have evaluated the viability, the adhesion and the proliferation of human adipose-derived stem cells (hADSCs) in association with poli(ε-caprolactone), PCL, a biodegradable polyester, for future application in regenerative medicine. After the Local Ethical Committee approval, human adipose tissue was minced and digested at 37°C in 3 mg/ml collagenase type I, for three hours. Primary cells were cultured in growth medium (10% FBS, 1 ng/ml bFGF and 1% antibiotics) at 37°C and 5% CO_2. Characterized hADSCs, (CD44+, CD105+, STRO1+ and CD45-), were plated on PCL film to evaluate fibronectin adhesion protein and cytoskeleton, by immunocytochemistry, using laser scanning confocal microscopy. Cell viability and proliferation were analysed by Acridine Orange (AO) staining and MTT assay on days 2, 4, 7, 10, 13, 16 after cell seeding on PCL film at appropriate density (5 x 10^3). Statistical analysis was performed by linearity test. No qualitative differences were found in cell adhesion and cytoskeleton fiber morphology between hADSCs grown on PCL film and hADSCs grown on polystyrene, used as control. MTT assay has measured a consistent growth of absorbance during all the studied period for hADSCs seeded on PCL (linear regression r^2= 0,86), suggesting that good cell viability was achieved on biomaterial. Cytocompatibility was also confirmed by AO staining microscopic observations. PCL construct has shown encouraging results in terms of cell adhesion and viability of hADSCs, opening new possibilities for future application in bone tissue engineering. Experiments are in progress in order to evaluate the osteogenic differentiation process of hADSCs on PCL.

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P183

Extracellular ATP and PTH Work in Concert to Inhibit the Sclerostin Expression by Osteosarcoma Cell Lines

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Sclerostin is expressed by SOST in osteoblast/octeocyte lineage cells. Sclerostin is anti-anabolic for bone formation through inhibition of the WNT signalling pathway. The aim of this study was to determine how SOST expression is regulated by ATP and PTH at different stages of osteoblastic development. Three human osteosarcoma cell lines representing different stages of osteoblast differentiation were used (MG-63 the least differentiated, TE-85 intermediate and SaOS-2 the most mature). Cells were grown in DMEM/10% FCS in 6 well plates until 90% confluent. Cells were serum deprived for 48 hours before treated with 10 μM ATP, 10 ng/ml PTH and 10 μM ATP + 10 ng/ml PTH, respectively and incubated at 37°C for 17 hours. mRNA was extracted and reverse transcribed to cDNA. Expression of sclerostin was monitored by qRT-PCR. Results were analysed using SPSS. In untreated cells, SaOS-2 cells had the highest expression of sclerostin ($p<0.05$) compared with MG63 and TE85. Expression of sclerostin was intermediate in TE85 cells but significantly higher than MG63 ($p<0.05$). Interestingly, sclerostin expression was barely detectable in MG-63 but abundantly expressed in SaOS-2 cells. Sclerostin transcripts were present in TE85 cells but at least 8 fold less than in SaOS-2 cells. In both TE85 and SaOS-2 cells, ATP, PTH and ATP+PTH treatment decreased the expression of sclerostin. The results reinforce previous reports on the relative differentiation status of these commonly used osteoblastic cell models. The robust expression of sclerostin in SaOS-2 suggests that this cell type represents a mature stage of the osteoblast / osteocyte lineage. The findings of this study shows that inhibition of sclerostin by ATP and PTH occurs at all stages of bone development. As ATP can be released by osteoblasts through mechanical stimulation, better understanding of the regulation of sclerostin should contribute to the development of new therapeutic approaches for preventing bone loss.

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Mechanical Stress Analysis and Osteoblastic Induction on Calcium Phosphate-Hydroxyapatite Cements

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Calcium phosphate biomaterials have been widely used as bone substitute materials in clinical applications due to their good biocompatibility and osteoconduction. However, they are usually plagued by low inherent mechanical properties. During this study we synthesised and analysed nanostructured calcium phosphate bone cements based on α-tricalcium phosphate (α-TCP) reinforced with nanoneedles of hydroxyapatite (HAP) (1, 3, 5, 10 and 20%) with strictly controlled porosity so as to optimise both mechanical properties and osteoblast nesting. Scanning electron microscope (SEM) images showed that the surface microstructure of the cements after hardening was composed of plate-like crystals. The compressive strength of pure cements was at 34.3 ± 1.7 MPa while in the case of composite cements is strongly depended upon the HAP content. By increasing the HAP content until 20 % the compressive strength drastically deceased until 4.9 ± 0.2 MPa which was within the cancellous bone ranges between 1.5 and 45 MPa. We further

analyzed the ability of the new biocements to induce the proliferation and survival of the pre-osteoblast cell line MC3T3E1 through DNA and protein quantification. In addition, cell morphology was observed with SEM after 1 and 7 days of culture. Moreover, the osteoblastic induction was quantified by alkaline phosphatase (ALP) activity measurements and the morphology of the cells with SEM after 14 days of culture in the presence of the osteogenic factors ascorbic acid and β-glycerophosphate. Our results indicate that biocements with certain ratio of α-TCP-HAP composite are tolerated as well as pure α-TCP with regard to their osteoinductive and osteogenic properties. In conclusion, pure or HAP loaded calcium phosphate bone cements are suitable for low stress loading locations and display similar biocompatibility.

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Toxicity and Metabolic Evaluation of Oestradiol, Coumestrol and Resveratrol on Osteoblast-like MLO-A5 and Osteocyte-like MLO-Y4 Cell Lines

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Phytoestrogens have been widely used by women as alternative hormone replacement therapy (HRT) during menopause. However, despite of several studies performed with phytoestrogens (PE), their risks and benefits are not yet well established. The identification of the molecular mechanisms of PE effects and their toxicity are required for a safe and appropriate therapeutic administration. We propose here to assess the effects of two distinct PEs coumestrol (COU) and resveratrol (RESV), when compared with Oestradiol (E2) on cell bioenergetics. We hypothesise that mitochondrial bioenergetics impairment in osteoblasts and/or osteocytes may be associated with the osteoporotic profile during E2 reduction, and can be recovered after HRT. Additionally, we tested the toxicity of those compounds on two murine osteoblast- and osteocyte-like cell lines. MLO-Y4 and MLO-A5 cell lines were seeded and screened in terms of cell viability, cell cycle, as well as extracellular acidification rate (ECAR) and oxygen consumption rate (OCR) by using a XF^{e}96 Extracellular Flux Analyzer. 24 hours prior to each assay cells were incubated with oestradiol, coumestrol and resveratrol at 1 μM concentration. Oestradiol, coumestrol and resveratrol had no toxicity in both cell types, and did not alter cell cycle. Interestingly, when we reduced the serum in the medium (from 10% to 2%) resveratrol decreased the apoptotic peak in MLO-A5 (compared with the control). MLO-A5 presented a notorious glycolytic profile, and in increasing order, E2, COU and RESV increased the ECAR parameter after the addition of glucose. The oxygen consumption analysis during mitochondrial stress tests in MLO-Y4 showed a slight increase after FCCP addition. No changes in media acidification were observed. The putative protective effect exerted by E2 and PEs is probably related to a positive influence on mitochondrial capacity and bioenergetics. MLO-A5 cell line showed a clear glycolytic profile, slightly increased in the presence of those PEs. MLO-Y4 cells seemed less susceptible to the effects of the compounds. At the selected concentrations, no toxic effects were observed. Although preliminary, the results contribute to understanding how the three selected PEs interfere with osteoblast and osteocyte-like cells.

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CELL BIOLOGY: OSTEOCLASTS AND BONE RESORPTION

P186 (OP27)

P187 (OP28)

Abstract withdrawn

P188

Unfolded Protein Response Mediator, the IRE1α-XBP1 Pathway Promotes Osteoclast Differentiation

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Background: Unfolded protein response (UPR) is a cellular stress response that is involved in the quality control of protein folding in the ER. We and others have recently shown that UPR is induced during bone formation and plays essential roles in promoting the differentiation and maturation of osteoblasts. In the present study, we aimed to clarify the potential roles of the IRE1α-XBP1 pathway, one of the major branch of UPR pathways, in the regulation of osteoclast differentiation.

Method and Results: We first performed in-vitro osteoclastogenesis experiments and found that the IRE1α-XBP1 pathway is temporarily activated during osteoclast differentiation. To explore the potential roles of the IRE1α-XBP1 pathway in osteoclastogenesis, we generated *Ire1α^{Mx1cre}* mice, in which the *Ire1α* locus can be excised in hematopoietic cells by injecting Polyinosinic-Polycytidylic acid. Excision was induced on day 10 after birth. The mice were sacrificed at 9-wk-old and subjected to uCT analysis and bone histomorphometry. The analyses showed a significant increase in bone volume in *Ire1α^{Mx1cre}* mice compared with wild type

controls due to a decrease in osteoclast number and activity, indicating that IRE1α functions as a positive regulator of osteoclastogenesis. Consistently, bone marrow transplantation experiments showed that wild type recipient mice transplanted with IRE1α-deficient bone marrow cells exhibited higher bone volume than those transplanted with wild type bone marrow cells. Transcriptional analysis revealed that IRE1α-deficient osteoclast precursors are defective in inducing *Nfatc1*, the master regulator of osteoclast differentiation. Most importantly, we found that XBP1, a transcription factor that is activated by IRE1α, binds to the promoter of *Nfatc1* gene and promotes its transcription. These observations indicate that the IRE1α-XBP1 pathway is a novel regulator of *Nfatc1* transcription.
Conclusion: The present study shows that the UPR mediator, the IRE1α-XBP1 pathway regulates osteoclast differentiation by promoting the transcription of *Nfatc1.*
Disclosure: The authors declared no competing interests.

P189
Inhibition of a Cholesterol Regulator, Srebp2, Prevents RANKL-Induced Bone Loss
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Clarification of the molecular mechanisms underlying osteoclastogenesis provides us new insights into both the physiology of bone metabolism and the pathophysiology of bone diseases. Recent studies revealed that osteoclastogenic Transcription Factors (TFs) sequentially and co-operatively facilitate the expression of osteoclast genes and determine osteoclast identity. However, there are still numerous other unidentified TFs involved in osteoclastogenesis. Recently, we have identified novel osteoclastogenic TFs including *Zscan10, Atf1, Nrf1,* and *Srebf2* by using a novel genome-wide approach, DNase-seq. However, physiological and pathological functions of these TFs in bone metabolism remain unclear although osteoclastogenesis was impaired by knockdown of these factors in primary osteoclasts. Among them, we focused on Srebp2 encoded by *Srebf2*, one of the master regulators of cholesterol regulation, because several studies reported a strong relationship between osteoclastogenesis and cholesterol homeostasis. To investigate the *in vivo* functions of Srebp2, RANKL-treated mice were administrated with an inhibitor of Srebp2, fatostatin. The mice were treated with fatostatin every day from 2 days before RANKL-injection, and the mice were sacrificed at 48 hrs after RANKL-injection. Then we performed micro-computed tomography (μCT) analysis. From the results of μCT analysis, fatostatin treatment rescued the reduction of trabecular bone volume, and trabecular number and the increase of trabecular separation in RANKL-treated mice. Bone histomorphometric analysis revealed that fatostatin treatment reduced the number of osteoclast in RANKL treated mice. Moreover, fatostatin treatment inhibited osteoclast differentiation *in vitro*. These results suggested that fatostatin might affect osteoclast differentiation mediating through Srebp2 inhibition and might prevent RANKL-induced bone loss *in vivo*. Taken together, our studies demonstrated that we succeeded in identifying Srebp2 as a novel transcription factor regulating osteoclastogenesis and inhibitors of Srebp2 might be a potential therapeutic strategy for osteoporosis. Further studies on Srebp2 functions will uncover more precise molecular mechanism underlying relationship between cholesterol homeostasis and bone metabolism.
Disclosure: The authors declared no competing interests.

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Monocyte Chemotactic Protein-1 (MCP-1) is a Key Regulator of Osteoclastogenesis
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Monocyte chemotactic protein-1 (MCP-1) plays a critical role in leukocyte recruitment and activation during inflammation, and has the highest level of gene induction in bone following anabolic PTH treatment1. MCP-1 is also specifically regulated during bone remodelling, activated to repair stress fracture2. We hypothesised that MCP-1 is a necessary regulator of osteoclastogenesis required for skeletal repair and remodelling. We used the ulnar stress fracture model, allowing scrutiny of focal remodelling with a known time course and precise anatomical location. Within 4 hours of stress fracture initiation, we observed significant increases in MCP-1 gene expression ($P < 0.01$), followed by increased serum levels within 24h ($P < 0.05$). To test our hypothesis, we used a plasmid DNA encoding a dominant negative mutant of MCP-1 (7ND) to specifically inhibit MCP-1 in vivo. Stress fracture was created in the right ulna of Wistar rats using cyclic end-loading. Unloaded animals were used as a control. 24 h prior to loading, 7ND plasmid vector, saline or empty vector control (pcDNA3.1), were injected in the thigh muscle to overexpress 7ND protein, effecting its secretion into systemic circulation. Rats were euthanised 4h (n=5/group) or 2 weeks (n=10/group) after loading for gene expression or histomorphometric analysis, respectively. MCP-1 gene expression (qPCR) increased significantly in untreated ulnae (saline and empty vector controls) within 4h of loading ($P<0.001$). This increase was abolished by 7ND treatment. At 2 weeks, there was a profound suppression of osteoclast number (61%), resorption area (50%) and new bone formation (60%) in basic multicellular units initiating remodelling of the stress fracture ($P < 0.05$). Conversely, 7ND treatment had no effect on formation of periosteal woven bone. MCP-1 is markedly upregulated by stress fracture, but also by intermittent PTH treatment. We therefore conclude that MCP-1 is a critical regulator of osteoclastogenesis during initiation events of bone remodelling.
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IL-8 is an Alternative Pathway for Osteoclastogenesis

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Physiologic bone resorption is regulated by macrophage colony stimulating factor (m-CSF), essential for osteoclast survival and receptor activated nuclear factor kB ligand (RANKL). Interestingly, we and others have demonstrated that alternative pathways of osteoclastogenesis are active under a variety of pathophysiologic situations, such as inflammatory arthritis and tumour osteolysis. In breast cancer bone metastasis, osteoclastogenesis is induced by tumour-derived agents such as Interleukin-8 (IL-8). Indeed, serum IL-8 levels are significantly correlated with increased bone resorption in breast cancer bone metastasis patients. Since no differences in osteoclast behaviour are apparent in normal or pathologic conditions, we hypothesised that RANKL and IL-8 signalling converge. First, no additive or apparent synergy was observed when human osteoclast precursors were differentiated with RANKL or IL-8. Next, western blot analysis of lysates from differentiated human osteoclasts treated with IL-8 resulted in phosphorylation of NF-kB, ERK, AKT and P38, all known RANKL-induced mediators of osteoclastogenesis. Furthermore, we determined the bone phenotype of novel IL-8 Tg mice that contain a bacterial artificial chromosome encompassing the entire human IL-8 gene, including all the endogenous regulatory elements. These mice have detectable IL-8 levels in serum and bone marrow supernatants >7.5pg/ml at baseline (uninduced) and up to ~5000pg/ml when stimulated using LPS (5mg/kg) and a low bone mass by microCT. *Ex vivo* bone marrow cultures from IL-8 Tg mice showed significantly decreased numbers of osteoclasts as well as a significant decrease in osteoblast recruitment and mineralisation, compared with age-matched WT mice. The decreased activity and number of osteoclasts and osteoblasts in *ex vivo* cultures explains the low bone mass phenotype observed *in vivo.* In summary, these data suggest that signals responsible for human osteoclastogenesis and bone resorption are shared between IL-8 and RANKL. Understanding the dynamics of pathologic osteoclast differentiation is critical for the development of effective therapeutic strategies.

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Lysosomal Associated Membrane Protein-2 (LAMP-2) is Involved in Osteoblastic RANKL Signalling

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Multinucleated osteoclasts are specialised cells with the capacity to resorb bone. To perform this activity, the osteoclast has a unique membrane area, the ruffled border, where protons are released to lower the pH. The membrane of the ruffled border is formed by fusion of lysosomal vacuoles and thus resembles the membrane of a lysosome. The composition of the lysosomal membrane is different from all other membranes due to an extreme high carbohydrate content, characteristic phospholipids, the presence of cholesterol, vacuolar proton ATPase and the membrane proteins LAMP-1 and 2. The latter proteins constitute 50% of the total amount of proteins of the membrane. Since it is known that the expression of LAMP-2 is very high in the ruffled border membrane, we investigated whether this protein plays a role in the formation of osteoclasts and/or in resorption. Bone marrow cells and osteoblasts were isolated from 6 weeks old wild type and LAMP-2 deficient male mice (approved by the Animal Welfare Committee, VU University Amsterdam). The bone marrow cells were cultured with M-CSF and RANKL for 6 days or co-cultured with osteoblasts for 21 days. Gene expression of RANKL was measured by qPCR. The presence of RANKL at the cell-membrane was investigated by FACS analysis.

We found in the cultures with M-CSF and RANKL more osteoclasts in the LAMP-2 deficient cultures. These osteoclasts contained also more nuclei per cell. Surprisingly, in co-cultures of osteoblasts isolated from LAMP-2 deficient mice with bone marrow cells, osteoclast formation was completely absent. Immunohistochemical staining of osteoblasts showed a comparable expression of RANKL between wild-type and LAMP-2 deficient osteoblasts. However, FACS revealed that RANKL expression on the plasma membrane was strongly reduced (WT 51% and LAMP-2 deficient cells 19%). Our data strongly suggest that LAMP-2 plays a crucial role in intracellular transportation of RANKL to the plasma membrane.

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Homer2/Homer3 Modulate RANKL-Induced NFATc1 Signalling in Osteoclastogenesis

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Ca^{2+} signalling and NFATc1 activation are essential for RANKL-induced osteoclastogenesis through the induction of Ca^{2+} oscillation, calcineurin activation, and translocation of NFATc1 into the nucleus. Homer proteins are scaffold proteins and have been proposed to modulate the multiple Ca^{2+} signalling channels and proteins, including inositol

1,4,5-triphosphate receptors, ryanodine receptors, transient receptor potential channels, and NFAT family of transcription factors in skeletal muscle cells and T cell. However, the role of Homer proteins in Ca^{2+} signalling during osteoclast differentiation is not well understood. In the present work, we investigated the role of Homer proteins (Homer2 and Homer3) on RANKL-induced Ca^{2+} signalling in osteoclasts using Homer2/Homer3 (Homer2/3) double-knockout (DKO) mice. Deletion of Homer2/3 markedly decreased the bone density of the tibias. In contrast, Homer2/3 deletion did not affect osteoblast formation and RANKL-induced Ca^{2+} oscillation. Forty-eight hours after RANKL treatment, there was a higher level of NFATc1 protein expression and significantly increased translocation of NFATc1 into the nucleus during osteoclastogenesis in the Homer2/3 DKO bone marrow-derived monocytes/macrophages (BMMs). Notably, the interaction of Homer proteins with NFATc1 inhibited its interaction by RANKL treatment, but restored by cyclosporine A treatment to inhibit the binding between NFATc1 and calcineurin in wild-type osteoclasts. In addition, RANKL treatment of Homer2/3 DKO BMMs significantly induced formation of multinucleated cells. These results suggest that Homer2/3 interact with NFATc1 and modulate the NFATc1 pathway in RANKL-induced osteoclastogenesis.
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Evidence that the Captured "Syncytin" Retroviral Envelope Genes Contribute to Fusion in Mouse Osteoclasts

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Syncytin-A and -B, envelope genes of retroviral origin, have been shown to contribute independently to the formation of the two syncytiotrophoblast layers during mouse placenta formation. In addition to this important role in placenta formation, syncytin-A and -B expression has been described in other tissues, and their highly fusogenic properties suggested that they might be involved in the fusion of other cell types. In order to elucidate the potential role of the syncytin-A and -B in osteoclast fusion and in overall bone metabolism/homeostasis, we analysed the bone phenotype of the syncytin-B knock out mice both *ex vivo* and *in vivo*. We first demonstrated by qPCR that both syncytin-A and -B are expressed during mouse splenocytes differentiation into osteoclasts. These genes are expressed through 14 days of culture, but the expression level decreases with differentiation. In the splenocyte and marrow macrophage cultures from syncytin-B KO mice, a reduced index of fusion and number of multinucleated osteoclasts are observed without impacting the osteoclastic resorption. Syncytin-B was expressed in TRAP+ cells of the periosteum at E16.5. The bone phenotype of the syncytin-B KO mice revealed no difference between the WT and KO littermates in term of BMD (at 6, 12, and 24 weeks of age), BV/TV (6 and 24 weeks of age) and D-pyridinolin/creatinuria (at 6 weeks). These results are in accordance with the *ex vivo* results showing a decrease of the fusion index without effect on the osteoclast function. Altogether the results suggest that although essential for placenta formation, syncytin-B is not essential to the maintenance of the bone homeostasis while it is expressed in osteoclast precursors and plays an early role in osteoclast fusion.
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Development of a New Immunological Assay for Tartrate Resistant Acid Phosphatase Isoform 5b (TRAcP 5b)

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Tartrate Resistant Acid Phosphatase (TRAcP) 5b is secreted exclusively by osteoclasts (cells responsible for resorbing bone). The level of TRAcP 5b in serum is therefore considered useful as a measure of osteoclast abundance and as an indirect indicator of bone resorption rate. Measurement of TRAcP 5b in serum is complicated due to the presence of a 10-fold higher concentration of TRAcP 5a, which is secreted by macrophages and dendritic cells. The two forms differ only in their glycosylation pattern and the removal of a 14 – 16 amino acid peptide from TRAcP 5b. Currently there is no automated immunoassay available for measurement of TRAcP 5b in human sera, so testing has been restricted to manual based assays. This work describes the initial performance assessment of the new IDS-iSYS TRAcP 5b (BoneTRAP®) assay. The TRAcP 5b assay developed on IDS-iSYS multi-discipline automated system has a measuring range from 0.5 to 14.0 U/L. The assay sensitivity is reported as limit of blank < 0.3 U/L; limit of detection: 0.5 U/L; and limit of quantitation ≤ 1.0 U/L. The total assay precision, as measured across 20 days is calculated as ≤ 12.0%. The assay shows a good correlation with Quidel MicroVue TRAP 5b assay yielding the following regression equation y (iSYS) = 1.02x (Quidel) + 0.18 with r2=0.95 (n=72). These results indicate the fully automated TRAcP 5b assay developed on the iSYS system is a sensitive and precise immunoassay. The first fully automated TRAcP 5b assay may provide a useful tool to assist in the diagnosis of osteoporosis and metabolic bone diseases including that associated with renal failure. Furthermore, the availability of a fully automated solution for detection of TRAcP 5b would simplify its measurement, increasing the potential for further research to be conducted into the involvement of TRAcP 5b in metabolic bone diseases.
Disclosure: The authors declared no competing interests.

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Osteoblast-Specific Overexpression of $G\alpha_{11}$ Protein Promotes Osteoclastogenesis and Leads to Enhanced Responses to Antiresorptive Therapy

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Background: $G\alpha_{11}$ is a member of the Gq protein family that mediates phospholipase C-dependent signalling downstream of G protein-coupled receptors. We previously demonstrated that dexamethasone increases $G\alpha_{11}$ expression in osteoblasts and can enhance parathyroid hormone signalling through the phospholipase C pathway. Using transgenic mice that overexpress $G\alpha_{11}$ in osteoblasts (G11-Tg) we showed that G11-Tg mice were osteopenic with a greater susceptibility to fracture as a result of reduced trabecular bone formation rate and increased osteoclast numbers and occupation surfaces. G11-Tg BMSC cultures had increased expression of osteoclast induction genes, *M-csf* and *Rankl* (*Bone* 2014 59:211). Our current study aims to investigate if $G\alpha_{11}$ overexpression induces osteoclastogenesis and bone resorption *in vitro*, and the effects on responses to antiresorptive therapy *in vivo*.
Methods: BMSCs from WT and G11-Tg mice were co-cultured with WT spleen cells on cell culture plates or dentin discs in the presence of 1α,25-dihydroxyvitamin D_3 to induce osteoclast differentiation. Osteoclastogenesis was evaluated with TRAP staining and measurement of resorption pits. To determine how G11-Tg mice respond to antiresorptive therapy, 8-week-old mice were treated with pamidronate (3 mg/kg weekly) or vehicle control. After 6 weeks of treatment, bone phenotypes were assessed by DEXA, microCT and histomorphometry.
Results: BMSC-osteoclast co-cultures derived from G11-Tg mice showed a significant increase in osteoclast number and size. Pamidronate treatment rescued the osteopenic phenotype of G11-Tg mice by returning femoral and L6 vertebral BMD to WT basal levels. Pamidronate had no effect on WT BMD. Histomorphometry of proximal tibial metaphyses showed significant improvement in trabecular bone volume in G11-Tg mice compared with WT.
Discussion: Our data suggest that osteoblast-specific overexpression of $G\alpha_{11}$ promotes osteoclastogenesis in the local bone environment and that osteoclasts are key mediators of trabecular bone loss in G11-Tg mice. We are currently elucidating how $G\alpha_{11}$ overexpression affects bone formation with anabolic therapies.
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Assessing Differences in Bone Affinity and Skeletal Retention of Fluorescently-labelled Bisphosphonates Relative to other Nitrogen-Containing Bisphosphonates in Rats

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The antiresorptive mechanism of action of nitrogen-containing bisphosphonates (N-BPs) is multifactorial. It has become clear that the bone affinity which targets BPs to bone, and inhibition of farnesyl diphosphate synthase (FPPS) within osteoclasts and osteoclast precursor cells are the main determinants of their potency to inhibit resorption. Fluorescent-BPs provide information on localisation of BPs in cortical and cancellous bone, as well as penetration at bone surfaces and within osteocyte canaliculi. We further evaluated the relative mineral affinity of both parent BPs and fluorescent-BPs by measuring their binding on HAP columns and disks. Mineral affinity was determined relative to risedronate (RIS = 1.0). Zoledronate (ZOL) had higher affinity than RIS, while minodronate (MIN) was similar. The addition of fluorescent groups to RIS can increase (rhodamine 2 (RhR) = 1.45) or decrease (alexafluor 647 (AF647) = .75) mineral affinity relative to RIS in the order: ROX-RIS > ZOL > RhR-RIS > RIS = MIN > fluorescein (FAM)-RIS = 800CW-ZOL > FAM-ZOL > AF647-RIS > deoxyRIS > RisPC. The impact of bone affinity *in vivo* on 24 hour urinary excretion after iv administration was also studied. Compounds were simultaneously injected in rats in this first head to head study of N-BPs. Approximately 90% of the amount excreted within 24 hours of both parent and fluorescent-BPs was excreted within 4 hours. The excretion within a 24 hour period relative to RIS, in order from high to low *in vivo* retention, was: alendronate > ZOL > RIS > etidronate > MIN > Ox-14 > neridronate > ibandronate = FAM-RIS > clodronate = AF647-RIS >> RisPC. This data indicates that binding of BPs to HAP strongly influences their excretion profiles, inversely reflecting skeletal retention. The ability to alter bone affinity among BPs with varied fluorescent tags is a useful tool for exploring the pharmacology of BPs.
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Tensin 3 Activates Dock5 to Drive Podosome Organisation in Osteoclasts and Efficient Bone Resorption

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Osteoclasts resorb the bone matrix through a specific adhesion structure called the sealing zone, which is based on a belt of podosome. Whereas the architecture of individual podosomes is getting well understood, a lot remains to be uncovered regarding the molecular mechanisms driving podosome organization into superstructures such as the osteoclast podosome belt. We showed that Dock5, an exchange factor for the small GTPase Rac is essential for podosome organisation in osteoclasts and then for bone resorption in vitro and in vivo (JBMR, 2011; Dev Biol 2014). We also demonstrated that systemic administration of an inhibitor of Dock5 protects the mice against pathological bone loss while preserving bone formation (Nature Communications in press). To understand how Dock5 signalling pathways control podosome belt formation, we performed proteomic analyses and identified Tensin 3 as a partner of Dock5 in osteoclasts. Confocal and 3D-SIM super-resolution microscopy revealed that Dock5 and Tensin 3 are not associated with individual podosomes. By contrast, they colocalise in the podosome cloud region when these assemble into a belt, standing further from the plasma membrane than vinculin. Suppression of Tensin 3 in osteoclasts destabilises podosome organisation and affects bone resorption. At the molecular level, we found that binding to Tensin 3 to Dock5 strongly increases its exchange activity towards Rac. Our results suggest that binding of Dock5 to Tensin 3 allows efficient activation of Rac to ensure the assembly of podosomes into a belt, the basal architecture of the bone resorbing apparatus of osteoclasts.

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Myristoleic Acid Inhibits Formation of Multinucleated Osteoclasts and Bone Resorption Activity by Suppressing Activation of Src and Pyk2

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Cytoskeletal changes in osteoclasts (OC) such as formation of actin ring or focal adhesion are an essential process leading to bone destruction. Therefore, some kinases involved in this process involving Src, integrin-linked kinase (ILK), or focal adhesion kinase (Pyk) may play a pivotal role in massive cytoskeletal change of OC, thereby destructing bone. It has been well appreciated that in order to for these kinases to be working on membrane anchoring of these kinases via myristoylation or phosphorylation is of importance. Myristoleic acid is an omega-5 fatty acid obtained from the plant seeds of the *Myristicaceae*, or biosnynthesised from myristic acid by the delta-9 desaturase. We observed that myristoleic acid inhibited RANKL-induced osteoclast formation, especially, at later stages and significantly attenuated phosphorylation of c-Src or Pyk *in vitro*. When myristoleic acid was co-administered with soluble RANKL into mice substantial degree of bone loss was prevented by myristoleic acid. Bone dissection clearly revealed that TRAP+ OC were significantly diminished with the treatment of myrostoleic acid. On the other hand, myristoleic acid treatment per se neither did block nor induce a number of common OC differentiation or maturation markers including c-Fos, NFATc1, DC-STAMP, integrin αv and integrin $\beta 3$ *in vitro*. ALP assay suggested that myristoleic acid did not give rise to an osteogenic potential. Interestingly, myristoleic acid substantially blocked bone resorption. Taken together, our data suggest that myristoleic acid is capable of blocking formation of multinucleated OC and bone resorption likely through suppressing activation of Src and Pyk2.

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P200

Distinct Roles of HIF-1α and HIF-2α in Osteoblast and Osteoclast Function

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Although the possible roles of hypoxia have been demonstrated extensively, much less is known about the regulatory mechanisms of HIF (hypoxia-inducible factor) families on osteoporosis. We undertook this study to explore the distinct roles of HIF-1α and HIF-2α in the regulation of bone mass. The role of HIF-1α and HIF-2α was investigated by overexpression or knockdown analysis. Both HIF-1α and HIF-2α significantly increased *Rankl* expression during osteoblast differentiation. In addition, RANKL-induced osteoclast differentiation was clearly regulated by HIF-2α (not by HIF-1α). We found that this regulation was mediated by regulation of osteoclast fusion-related genes such as *DC-stamp* and *OC-stamp*. We further examined the *in vivo* role of HIF-1α and HIF-2α in linking osteoblast and osteoclast functions on osteoporosis in ovariectomised-knockout mice lacking HIF-1α or HIF-2α. HIF-1α or HIF-2α deficiency in mice reduced the development of ovariectomy-induced bone loss compared with their wild type mice. Collectively, these results indicate that HIF-1α and HIF-2α have a pivotal role in the regulation of bone mass through distinct mechanisms.

Disclosure: The authors declared no competing interests.

P201

Osteoclastogenesis-Insensitivity, a Phenomenon that is Induced by M-CSF Priming and Prevented when Osteoclast Precursors Adhere to Bone

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Osteoclasts and macrophages share progenitors that must receive decisive lineage signals driving them into their respective differentiation routes. Macrophage colony stimulation factor (M-CSF) is a common factor used for both lineages; bone is likely the stimulus for osteoclast differentiation. To elucidate the effect of both factors, mouse bone marrow precursor myeloid blasts were pre-cultured with M-CSF on plastic and on bone and the effect of osteoclast formation, gene and protein expression and bone resorption were assessed. M-CSF priming for three or four days prior to stimulation with M-CSF in combination with RANKL resulted in a complete loss of osteoclastogenic potential on tissue culture plastic. Such M-CSF primed cells expressed the receptor RANK, but lacked the crucial osteoclastogenic transcription factor NFATc1. This coincided with a steeply decreased expression of the osteoclast genes TRACP and DC-STAMP ($p<0.05$), but an increased expression of the macrophage markers F4/80 and CD11b. Compellingly, M-CSF priming on bone accelerated the osteoclastogenic potential: M-CSF primed cells that had received only one day M-CSF and RANKL and were grown on bone already expressed an array of genes that are associated with osteoclast differentiation and these cells differentiated into osteoclasts within 2 days. Osteoclastogenesis-insensitive precursors grown in the absence of bone regained their osteoclastogenic potential when transferred to bone. This implies that adhesion to bone dictates the fate of osteoclast precursors. Common macrophage-osteoclast precursors may become insensitive to differentiate into osteoclasts and regain osteoclastogenesis potential when in contact with or in the vicinity of bone.
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P202

The Cellular Basis for Osteoclast Fusion

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Bone resorption is achieved through the work of Osteoclasts. Osteoclasts are myeloidic cells closely related to macrophages. During differentiation some osteoclasts might undergo fusion to form a giant multi-nucleated cell (polykaryon). Although in recent years new proteins regulating osteoclast fusion have been discovered the cellular characteristics of osteoclast progenitors undergoing fusion is poorly defined. Using time lapse microscopy we show that in a given population of osteoclast progenitors some cells are more prone to fuse, assuming the role of fusion epicenters, which attract other, more passive, cells. Furthermore, while rate of fusion events involving polykaryons increases throughout the differentiation period, fusion events involving only mono-nucleated cells remains constant, suggesting a population of predisposed cells leading the fusion process (founders) and a population of cells being led (followers). In order to determine the distribution of founder cells we cultured osteoclast progenitors on micro-wells and scored for polykarions as cells with three or more nuclei. Our statistical analysis show that the fraction of founder cells in a given population of RAW264.7 differentiating osteoclasts is about 4% of the total progenitor population. Using a system we developed which allows us to track the origin of nuclei of two differently treated populations, we show that founder enriched RAW264.7 and primary osteoclast progenitor population (primed by 48 hours exposure to RANKL) can undergo fusion with cells which are not pretreated with RANKL, demonstrating that fusion is not directly linked to differentiation induced by the known cytokines. These results and observations suggest the existence of a founder-follower mechanism. Understanding this mechanism may provide essential insights regarding the physiological role of fusion in normal, developmental and pathological states of bone.
Disclosure: The authors declared no competing interests.

P203

Inhibitory Effects of Carvacrol on Osteoclast Formation, Function and Survival

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Bone loss is associated with a wide array of diseases such as osteoporosis, cancer and chronic periodontitis. One of the common factors observed among these diseases involves elevated osteoclast activity. Selective targeting of osteoclast formation is an effective approach to exacerbate bone loss. In this study, we investigated the effects of carvacrol, a monoterpenic phenol present in the essential oils of *Origanum vulgare*, *Thymus vulgaris* and *Carum copticum* on osteoclast formation and activity. The effects of carvacrol on osteoclastogenesis was assessed by analysing the following: cell viability, TRAP+ multinucleated osteoclast formation, actin ring formation, bone resorption and nuclear fragmentation assay. We found that treatment of RAW264.7 macrophages and human CD14+ monocytes with carvacrol suppressed RANKL-induced osteoclast formation in a dose-dependent manner without cytotoxicity. Carvacrol also inhibited the bacterial lipopolysaccharide (LPS)-induced osteoclast formation in RAW264.7 macrophages. Moreover, treatment of RAW264.7 macrophages with conditioned-media derived from MDA-MB-231 and MCF-7 human breast cancer cells resulted in osteoclast formation which was further perturbed by carvacrol. Carvacrol disrupted actin ring formation and bone resorptive function of osteoclasts. Intriguingly, exposure of mature osteoclasts to carvacrol lead to a reduction in osteoclast numbers. These cells showed nuclear fragmentation and condensation, a characteristic hallmark of apoptosis. Additionally, carvacrol did not decrease

cell viability of MC3T3-E1 osteoblast like cells. Together, these results suggest that carvacrol effectively blocks osteoclastogenesis induced under various pathophysiological stimuli and could be developed as a therapeutic agent.

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P204

Tartrate Resistant Acid Phosphatase 5a – a Coupling Factor Between Osteoclast and Osteoblast with Potential Growth Factor Activity?

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Tartrate- resistant acid phosphatase (TRAP) is a phosphatase highly expressed by osteoclasts (OCs). Also osteoblasts (OB)/ osteocytes (OCy) expresses TRAP.[1,2] In OC, TRAP is secreted as an enzymatically inactive proenzyme, TRAP5a, that processed by cysteine proteinases forms enzymatically active TRAP5b.[3] Conventionally, TRAP 5a has been regarded as a latent pro-form of TRAP 5b with no biological activity. Recently, this view has been challenged with the discovery that TRAP 5a, but not 5b, induces proliferation/differentiation in the mesenchymal stem cells. Also, TRAP overexpression leads to increased OB activity and cortical bone.[4] Additionally, TRAP induces proliferation in haematopoietic stem cells i.e. the lineage from where OC precursors are derived. To investigate the role of TRAP 5a on OB and OC we used cell culture, immunohistochemistry, cell cycle and morphological analysis in combination with MC3T3-E1, RAW 264.7 and TRAP overexpressing mice. Addition of TRAP 5a to MC3T3-E1 cells caused an increase in number of cells in S-phase. Moreover, TRAP 5a caused morphological changes consistent with alterations of the cytoskeleton. In addition, elevated levels of circulating monocytes were found in TRAP overexpressing mice. The study was approved by the Stockholm South Animal Ethical Committee (S159/01 and S235/04). Based on these data we suggest that TRAP5a is released into the resorption lacuna and thus could potentially function as a coupling factor between OC-OB to increase OB proliferation and activity. Additionally, we hypothesise that TRAP 5a might increase numbers of OC precursors. This work was funded by Swedish Research Council and funds from Karolinska Institutet.

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P205

Uptake and Vesicular Trafficking Dynamics of Degraded Bone Matrix in Osteoclasts

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Osteoclasts are large multinucleated cells exquisitely adapted to degrade bone matrix. Upon contact with bone, osteoclasts segregate their surface membrane into four polarised domains: 1) the sealing zone; 2) the basolateral membrane; 3) functional secretory domain (FSD) and; 4) the bone-apposed ruffled border (RB). The RB functions as the 'resorptive apparatus', serving as a release site for protons and osteolytic enzymes (i.e. cathepsin K) required to digest the mineral and organic phases of bone. At the same time, the RB facilitates the uptake and transcytosis of bone matrix by-products to the opposing FSD where they are expelled into the extracellular milieu. Although the crucial importance of vesicular trafficking between the RB and FSD during the functional bone resorption cycle is now well-established, the spatiotemporal dynamics of this process has not yet been appreciated in real-time. Here using confocal microscopy, we have monitored the intracellular uptake and trafficking dynamics of degraded bone matrix in osteoclasts actively engaged in bone resorption. Using fluorescently labelled bone substrates together with a panel of intracellular compartment markers we demonstrate that osteoclasts utilise multiple endo-lysosomal trafficking pathways to ingest degraded bone matrix particles at the RB. In addition, we show that osteoclasts employ, pseudopodal-like actin-rich projections to facilitate phagocytic bone uptake. Following phagocytosis, bone particles undergo further degradation and associate with both lysosomal and autophagic pathways before converging with the transcytotic pathway en route to the FSD. Overall, these studies provide new insights into the uptake, trafficking and dynamics of degraded bone matrix during the bone resorption cycle.

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P206

Effects of Platelet-Released Supernatants with and without Serum on Differentiation and Activity of Osteoclasts and Osteoblasts

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Platelet preparations are clinically applied to stimulate healing of oral tissue in regenerative dentistry. Platelets can modulate differentiation and activity of osteoclasts and osteoblasts. Research with different preparations of platelets is not conclusive. In the present study, we assessed if serum components modulate the effect of platelet preparations. In addition, we evaluated if collagen barrier membranes, which are clinically used in guided bone regeneration, can serve as carrier for platelet preparations. In murine bone marrow cultures, osteoclastogenesis was investigated in the presence of platelet-released supernatant (PRS) and serum containing PRS (SC-PRS). We quantified differentiation of osteoclasts based on the number of tartrate-resistant acid phosphatase (TRAP)-positive multinucleated cells (MNC), TRAP activity and resorption activity. In addition, viability and proliferation were tested. Osteoblastogenesis was assessed based on staining for alkaline phosphatase (AP). To assess mitogenic effects of collagen barrier membranes loaded with PRS and SC-PRS bioassays were performed and the release of platelet-derived growth factor (PDGF)-BB was measured. Our results show that PRS increases the number of TRAP positive MNC and their activity. SC-PRS decreased the number and activity of TRAP positive MNC. SC-PRS decreased formazan formation and ^{3}[H]thymidine incorporation of osteoclast progenitors. Our results on osteoblastogenesis indicate that PRS can decrease the number of AP-positive colonies while SC-PRS can increased osteoblast markers. Proliferation of osteoblast-like cells was stimulated by all preparations. Collagen barrier membranes loaded with PRS and SC-PRS released PDGF-BB and stimulated proliferation. In conclusion, activated platelets stimulate differentiation of osteoclasts, while serum containing preparations decrease differentiation of osteoclasts and increase differentiation of osteoblasts. Our findings suggest that serum components modulate the effects of platelet preparations on osteoclastogenesis and osteoblastogenesis. Future studies will reveal the impact of these preparations on guided bone regeneration.

Disclosure: The authors declared no competing interests.

P207

Inhibition of Osteoclast Differentiation by 1,25-Dihydroxyvitamin D_3 through mTOR Signalling Pathway

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1α,25-dihydroxyvitamin D_3 **(**1,25$(OH)_2D_3$) is a key molecule to maintain calcium homeostasis and bone metabolism. In addition to the role in calcium homeostasis and bone metabolism, 1,25$(OH)_2D_3$ directly inhibits osteoclast differentiation in human and mouse osteoclast precursors. However, the exact mechanism of 1,25$(OH)_2D_3$-induced inhibition on osteoclast differentiation remains largely unknown. Recently, mTOR signalling was reported to be involved in osteoclast differentiation. In this study, we showed the role of mTOR signalling pathway in 1,25$(OH)_2D_3$-induced inhibition on osteoclast differentiation. 1,25$(OH)_2D_3$ strongly inhibited osteoclast differentiation in mouse bone marrow cells and 1,25$(OH)_2D_3$-induced inhibition of osteoclast differentiation was significantly reversed by specific inhibitors of mammalian target of rapamycin (mTOR) signalling pathway, including rapamycin and torin 1. 1,25$(OH)_2D_3$ induced phosphorylation of p70S6 kinase and Akt, two targets of mTOR complex 1 (mTORC1) and mTORC2, respectively. In addition, 1,25$(OH)_2D_3$-induced inhibition of osteoclast differentiation was significantly reversed by Raptor small interfering RNA (siRNA), suggesting that mTORC1 is involved in 1,25$(OH)_2D_3$-induced inhibition of osteoclast differentiation. Our results indicate that 1,25$(OH)_2D_3$ inhibits osteoclast differentiation through mTOR signalling pathway.

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P208

TNF-α Antagonist Infliximab Inhibits Osteoclast Formation of Peripheral Blood Mononuclear Cells but does not Affect Periodontal Ligament Fibroblast Mediated Osteoclast Formation

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The inflammatory cytokine tumuor necrosis factor-alpha (TNF-α) is elevated in inflamed periodontal tissues and may contribute to periodontitis progression. TNF-α stimulates formation and activity of osteoclasts, the cells that cause alveolar bone degradation and subsequent tooth loss. We previously showed that TNF-α is elevated in co-cultures of periodontal ligament fibroblast (PDLF) and peripheral blood mononuclear cells (PBMC). Hence, TNF-α could be a determining factor in osteoclast formation in these cultures. To assess the role of TNF-α in periodontitis associated osteoclast formation *in vitro*, osteoclast formation was analysed in the presence of the anti-TNF-α therapeutic agent

infliximab in two culture systems: (i) PBMC in co-culture with periodontal ligament fibroblasts from controls and periodontitis patients, or (ii) with PBMC only. The highest level of TNF-α was found in supernatants at day 7 in co-cultures and declined at days 14 and 21 ($p < 0.001$). TNF-α was undetectable in cultures that received infliximab. The formation and activity of osteoclasts in co-cultures was not affected by infliximab. In contrast, infliximab in cultures of only PBMCs significantly reduced the formation of osteoclasts ($p < 0.01$). This reduction was accompanied by a decreased number and size of clustered cells, a step that precedes the formation of osteoclasts. Our study shows that the contribution of TNF-α to osteoclast formation is cell system dependent. It contributes to PBMC induced osteoclast formation, possibly by establishing stronger cell-cell interactions that precede osteoclast formation.

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P209

Osteoclast-Associated Receptor (OSCAR) Gene Expression and Protein Release by Human Peripheral Blood Derived Osteoclast Cells in Response to RANKL +/- TNF-α

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Osteoclast-associated receptor [OSCAR] is a co-stimulatory molecule involved in osteoclast differentiation. Increased protein expression is present in synovial tissues of Rheumatoid Arthritic joints and in soft tissues adjacent to sites of peri-implant osteolysis. Soluble OSCAR is detectable in serum and synovial fluid but its role in the regulation of bone erosion is not clear. This study assessed the effect of RANKL with/out TNF-αon the mRNA expression and protein release of OSCAR by human peripheral blood (PBMC)-derived osteoclasts. Human PBMCs pre-cultured with MCSF for 7 days were differentiated into osteoclasts with 10 ng/ml or 50 ng/ml RANKL over 10 days. TNF-α was added to 10 ng/ml RANKL treated cultures; and/or at 1 or 3 days post RANKL exposure. Media was changed every 3-4 days. After 7 days with RANKL; supernatants were assessed for OSCAR levels by ELISA, cells were stained for TRAP or RNA extracted for RT QPCR. After 10 days differentiation dentine slices were assessed for resorption pits by Scanning Electron Microscopy (SEM). Soluble OSCAR was produced by PBMC derived osteoclast cells in response to RANKL with and without TNF-α with 50ng/ml RANKL compared with 10 ng/ml. The highest levels of OSCAR were detected when TNF-α was added pre-RANKL and on day 1. Consistent with this, OSCAR mRNA expression was significantly higher when TNF-α was added pre-RANKL and on day 1 compared with TNF-α pre-treatment only ($p=0.0183$) and TNF-α from day 3 ($p=0.0381$). The greatest resorption was observed with the TNF-α pre-treatment and RANKL at 10 ng/ml, and this was significantly greater than RANKL 10 ng/ml ($p= 0.0370$) and RANKL 50 ng/ml ($p= 0.0499$) without TNF-α. TRAP positive cells, osteoclast gene expression and resorption pits were evident with all treatments confirming the presence of active osteoclasts. OSCAR expression and release by osteoclast cells is mediated by RANKL and TNF-α influenced by timing of exposure.

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P210

Novel Small Molecule Inhibitors of Human RANKL

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Receptor activator of nuclear factor-κB ligand (RANKL), a trimeric tumour necrosis factor (TNF) superfamily member, is the central mediator of osteoclast formation and bone resorption. Functional mutations in RANKL lead to human autosomal recessive osteopetrosis, whereas RANKL overexpression has been implicated in the pathogenesis of bone degenerative diseases such as osteoporosis. Following a forward genetics approach, we have recently shown that a novel loss-of-function allele of *Rankl* with a glycine-to-arginine susbstitution at codon 278, causes severe recessive osteopetrosis in mice due to inhibition of RANKL trimerisation. Notably, SPD304, a small molecule inhibitor of TNF trimerisation, also binds and inhibits RANKL, suggesting similar inhibitory mechanisms. However, SPD304 displays high cell toxicity. Based on the trimeric structure of RANKL and its interaction with SPD304, novel small molecules were designed to abrogate RANKL trimer formation and biological function while also displaying lower toxicity. Of the 72 SPD304-like derivatives synthesised and tested, 8 displayed complete inhibition of human RANKL-induced osteoclastogenesis at 5 μM without affecting the differentiation of the preosteoblastic MC3T3-E1 cells. Notably, these compounds were significantly less cytotoxic compared with SPD304 as shown by MTT. Moreover, the most effective small molecule inhibitors dissociated human RANKL trimers as shown by cross linking and western blot and suppressed the activation of NFATc1, the master regulator of osteoclast formation. Our research identified potent small molecule inhibitors of human RANKL designed to target and block its trimerisation. The more effective inhibitors will be further evaluated *in vivo* using our unique human RANKL-expressing transgenic mouse models of osteoporosis.

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P211

Different Human Osteoclast Precursors Respond in Distinct Ways to IL-17A

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Background: Bone degrading osteoclasts (OCs) play an important role in several inflammatory skeletal disorders. They differentiate from the monocyte/macrophage lineage. In this lineage, three subsets have been identified; classical, intermediate and non-classical monocytes. The capacity of these subsets to differentiate into OCs and whether inflammatory cytokines influence this differentiation is unknown. The objective was to evaluate osteoclastogenesis of the monocyte subsets and the effect of inflammatory factor interleukin-17A (IL-17A) hereupon.
Methods: Monocytes were isolated from peripheral blood and sorted with flow cytometry based on CD14 and CD16 expression. The subsets were seeded onto plastic and bone. Differentiation was induced by osteoclastogenic medium containing 10 ng/ml M-CSF and 2 ng/ml RANKL, with or without 10 ng/ml IL-17A. After 17 days, OCs were visualised with TRACP staining and bone resorption was evaluated with Coomassie Brilliant Blue staining.
Results: All precursor subsets gave rise to OCs. IL-17A did not affect the total number of OCs in the classical subset, but a significant lower number was observed for the intermediate subset on both plastic and bone. The number of OCs derived from the non-classical subset was up-regulated on bone and an increased number of large OCs (>20 nuclei) was observed on plastic. Extensive bone resorption was only observed for the classical subset, independent of IL-17A.
Conclusion: Different subsets of human OC precursors from peripheral blood respond in distinct ways to IL-17A treatment and targeting of specific precursor subsets is a promising therapeutic approach for diseases associated with inflammatory bone loss.
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IL-1β Differently Affects Osteoclastogenesis of Distinct Subsets of Osteoclast Precursors

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Background: Osteoclasts are bone resorbing cells and treatment of bone diseases like rheumatoid arthritis have targeted this cell type. It is reported that distinct osteoclast precursor subsets like early blasts, myeloid blasts and monocytes respond differently to the osteoclastogenesis inducing cytokines M-CSF and RANKL. Whether these subsets respond also differently to the osteoclast stimulating inflammatory cytokine IL-1β is not known. Our objective was to investigate the effect of IL-1β on osteoclastogenesis by early blasts, myeloid blasts and monocytes.
Methods: Bone marrow cells were isolated from 6 weeks old male mice (approved by the Animal Welfare Committee of the VU University Amsterdam). Early blasts, myeloid blasts and monocytes were sorted using flow cytometry. Cells were cultured in the presence of M-CSF and RANKL for 4-6 days, without or with IL-1β (0.1-10 ng/ml), on plastic and bone, followed by TRACP staining to identify osteoclasts and Coomassie Brilliant Blue staining to visualise bone resorption. Gene expression was quantified using qPCR.
Results: Osteoclast formation on plastic was stimulated in early blasts and myeloid blasts whereas monocytes responded less to IL-1β. Very large osteoclasts (>20 nuclei) were formed in myeloid blast cultures whereas smaller (3-5 nuclei) osteoclasts were formed from early blasts. IL-1β caused a significantly increased bone resorption by osteoclasts generated from monocytes. TRACP and DC-STAMP were highly expressed by myeloid blasts, and IL-1RI is initially high expressed while IL-RII is low expressed in early blasts.
Conclusion: IL-1β differently affects osteoclastogenesis. Osteoclast formation is stimulated in early precursors like early blasts and myeloid blasts both on plastic and on bone. Monocytes hardly respond to IL-1β on plastic, but they did when seeded on bone. We propose that early blasts and myeloid blasts are the osteoclast precursors recruited during inflammation.
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Effects of Bisphosphonates on Osteoclastogenesis and V-ATPase Function of Osteoclasts *In Vitro*

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Background: The present study aimed to analyse the expression of osteoclastogenesis-related molecules and the

cellular location of the B2 V-ATPase subunit (B2 V-ATPase), which is related to resorption activity, in osteoclast precursors from mouse marrow cells treated with different bisphosphonates.
Methods: Tibia and femur marrow cells obtained from 30 days-old Balb/c mice were cultured in α-MEM supplemented with calcitriol onto the bone slices for 5 days. The bone substracts were previously soaked in α-MEM containing alendronate (AL), etidronate (ET) or zoledronic acid (ZA) or only in medium (V). The bone resorption was analysed by SEM, and the cells were incubated for TRAP, TUNEL or submitted to MTT assay. Additional cells were harvested from the surface of the bone substrate and the expression of osteoclastogenesis-related genes (RANK, RANKL, OPG, CSF-1 and CSF-1R) was analysed by qPCR. For B2 V-ATPase analysis, the cells were harvested from the bone surfaces and spun at $50x10^3$ rpm in an ultracentrifuge. Pellet and supernatant fractions were resolved in SDS-PAGE, blotted into nitrocellulose membrane and immunoblots were detected by electrochemiluminescence.
Results: V and ET specimens presented more bone resorption than AL and ZA. The V group presented more TRAP-positive cells with more than 5 nuclei than other groups. MTT values were higher in ZA group, while the number apoptotic cells was higher in ET. AL reduced the expression of CSF-1 and RANKL, while ZA reduced the expression of RANK and increased the expression of OPG. The B2 V-ATPase was found in the supernatant only in vehicles and remained bound to the pellet in the treated groups.
Conclusion: Therefore, AL and ZA inhibit osteoclastogenesis by different mechanisms, besides the effects on resorption activity. The reduced bone resorption may be related to the inhibited binding of B2 V-ATPase to the cell membrane by bisphosphonates.
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P214
Unexpected Effects of TNF-A IL-1β, and IL-6 on Murine Osteoclastogenesis
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Traditionally, interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α) have been regarded as classical proinflammatory and bone resorptive cytokines. Nevertheless, a few recent publications give evidence for inhibitory effects of TNF-α on osteoclastogenesis. The aim of this work was to analyse the effects of IL-1β, IL-6 and TNF-α on osteoclastogenesis and cathepsin K expression in primary murine bone marrow osteoclast cultures. Primary murine bone marrow cell cultures from 2-3 month old HIM: OF-1 mice were supplemented with 1,25 dihydroxy vitamin D_3 to induce osteoclatogenesis and combined or individually treated with the proinflammatory cytokines IL-1β, IL-6 and TNF-α. After a culture period of one week, cells were stained for tartrate resistant acid phosphatase (TRAP), mRNA levels of cathepsin K, RANKL and OPG were determined by real-time-PCR, and protein expression of cathepsin K was assessed by immunofluorescence staining. Combined treatment and treatment with the individual cytokines led to a significant decrease in the number of generated osteoclasts as assessed by TRAP staining, whereas protein expression and mRNA expression of cathepsin K were not influenced. However, there was as trend towards a lower cathepsin K mRNA expression in cultures individually treated with interleukin-6 or TNF-α, and a lower RANKL/OPG mRNA ratio in cultures individually treated with TNF-α. We conclude, that in our experimental setting the proinflammatory cytokines IL-1β, IL-6 and TNF-α decrease the generation of osteoclasts, but have not significant effect on mRNA or protein expression of cathepsin K.
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P215
ERK5 Activation is essential for the Differentiation of Preosteoclasts into Osteoclasts
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The 4B12 preosteoclast cells differentiate into osteoclasts upon the stimulation of M-CSF and sRANKL. We found that Erk5 is activated by M-CSF. Inhibition of MEK5 by BIX02189 or inhibition of Erk5 by XMP 8-92 blocked the osteoclast differentiation. MEK5 siRNA inhibited differentiation of 4B12 cells, confirming these results. Raw264.7D clone cells, which are monocytic cells, differentiate into osteoclasts after stimulation with sRANKL. Erk5 was activated without any stimulation in these cells. Inhibition of the Erk5 pathway by the inhibitors also blocked differentiation of these cells into osteoclasts. Moreover, induction of c-Fos was inhibited. Therefore, activation of ERK5 is required for induction of c-Fos. Taken together, activation of the Erk5 pathway is required for differentiation of preosteoclasts into osteoclasts through induction of c-Fos.
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CHONDROCYTES AND CARTILAGE

P224 (OP21)

P225 (OP33)

P226 (OP34)

P227 (OP35)

P228

Runx2 Regulates ST2 Expression in the Late Stages of Growth Plate Chondrocyte Differentiation

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The primary response gene ST2 (IL1RL1 or T1) was suggested as an early marker of differentiation in osteogenic cell lines. ST2 receptor (ST2L) plays an important role in regulating osteogenic potential of osteosarcoma cells as well as regulation of osteoclastogenesis. In this study we aimed to investigate expression, regulation and possible biological role of this gene in the growth plate chondrocytes which is responsible for long bone elongation. We confirmed expression of both ST2L and soluble ST2 (sST2) in murine chondrogenic cell line ATDC5 using reverse transcription PCR. Increased to strong ST2 expression was observed by immunohistochemistry at pre-hypertrophic and hypertrophic chondrocytes of the tibial growth plate of euthanised three week old mice. Surprisingly, consistent with these results we noted several fold upregulation of both ST2L and sST2 mRNA in the late stages of ATDC5 differentiation. ColX and MMP-13, markers of chondrocyte hypertrophy, were also significantly increased in these hypertrophic ATDC5 cells. Master transcription factor Runx2 which controls chondrocyte hypertrophy was shown to be upregulated at differentiated ATDC5 cells. Our results showed that Runx2 upregulation by cDNA transfection or downregulation by siRNA knockdown significantly increase or decrease ST2 expression, respectively. Our results clearly indicated that both ST2 splice variants are transcribed from proximal promoter and low sST2 mRNA is also produced from distal promoter in Runx2 overexpressing cells. *In silico* promoter analysis identified consensus Runx2 binding sites on distal and proximal promoters and electrophoretic mobility shift assay demonstrated binding of Runx2 to both promoters. These results were confirmed further by chromatin immunoprecipitation assay. Overall, our data suggest that Runx2 is involved in regulating enhanced ST2 expression in pre- and hypertrophic chondrocytes. Therefore, further investigations might lead to elucidate ST2 function in the highly organised cartilaginous growth plate and thus give a new insight into the process of longitudinal bone growth.

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P229

Enhanced Chondrogenic Potential of miR-221 and Slug Depleted Human MSCs

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Human Mesenchymal Stromal Cells (hMSCs)-based tissue engineering is regarded as a very promising approach for cartilage regeneration. Our work is aimed at identifying new molecules having a crucial role in determining MSCs fate, and targeting such regulators for the guidance of chondrogenesis in the absence of differentiating agents, such as TGF- . Recently, miR-221 and Slug transcription factor have emerged as anti-chondrogenic regulators. We investigated if inhibition of these factors by specific antagomiR or siRNA molecule could be sufficient to address hMSCs from Wharton's Jelly (WJMSCs) towards chondrogenesis, in the absence of TGF-β. We demonstrated by immunocytochemistry assays that miR-221 or Slug silencing increased the expression of the major cartilage protein Col2A1 and the master chondrogenic regulator Sox9, while decreased Col1A1 expression. Only Slug silenced WJMSCs were able to increase the expression of TRPS1, a positive regulator of chondrocyte differentiation. In addition, Slug inhibition determined a reduction in the levels of miR-221, and we identified by chromatin immunoprecipitation assay a specific region of the miR-221 promoter that is involved in the *in vivo* recruitment of Slug. By embedding miR-221 or Slug depleted bone marrow MSCs in alginate constructs, we also confirmed the stability of gene silencing for 28 days after combination with the scaffold. Taken together, our data demonstrate that miR-221 and Slug are functionally correlated in MSCs and that the silencing of these regulators is sufficient to induce differentiation towards the chondrogenic lineage, in the absence of TGF-β. The combination of engineered hMSCs with alginate preserved the efficiency of gene silencing, demonstrating the feasibility of this approach for the generation of tissue engineering constructs. On-going experiments are aimed at evaluating the ability of the engineered hMSCs to trigger cartilage reparative processes in vitro or *in vivo*, by using an experimental model of osteochondral defect.

Disclosure: The authors declared no competing interests.

P230

Osteoclasts Activate Chondrocyte Catabolism through S1P Production

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Background: Subchondral bone features accompany all stages of osteoarthritis (OA). We have previously demonstrated that high osteoclastogenesis and bone remodelling is observed at the initiation of OA, while inhibition of osteoclast

function prevents bone and cartilage catabolism in murine OA models. Our purpose was to evaluate how osteoclast-derived factors affect the chondrocyte metabolism and to further investigated the role of sphingosine 1 phosphate (S1P), an osteoclast-secreted molecule in chondrocyte metabolism and osteoarthritis.
Methods: Primary murine chondrocytes were cultured with conditioned medium of osteoclasts (Oc-M) or RAW cells (Raw-CM) to analyse the expression of catabolism and anabolism genes (RT-qPCR). Femoral head explants were cultured in the presence of Oc-CM to quantify matrix protein expression and proteoglycan content and further investigate the role of S1P released in Oc-CM in the presence of JTE-013, a S1P receptor 2 (S1PR2) antagonist.
Results: Oc-CM reduced the proteoglycan release in primary chondrocytes and activated MAPkinase pathway. Increased expression of catabolic enzymes (MMP-3, -13, Adamts-4,-5) was observed only with Oc-CM while reduction of expression of anabolic markers (Col2, ACAN, Sox9) was induced by both Oc-CM and Raw-CM. Oc-CM increased the chondrocytic expression of S1P receptors 1 to 4 and the inhibition of S1PR2 protected chondrocytes from degradation enzymes induced by Oc-CM. In joint explants, JTE-013 reversed proteoglycan loss and NITEGE expression induced by Oc-CM, and reduced proteoglycan release and expression of MMP-3 / MMP-13 by the chondrocytes. Our results indicate that S1P produced by osteoclasts promotes chondrocyte catabolism.
Conclusion: These data demonstrate that osteoclast-secreted factors disrupt the balance of chondrocyte metabolism through the production of S1P. Therefore, subchondral bone manipulations may affect chondrocyte function and OA.
Disclosure: The authors declared no competing interests.

P231
Disruption of NFI-C Causes Defects in Postnatal Cartilage Development
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The nuclear factor I (NFI) gene family encodes site-specific transcription factors essential for the development of a number of organ systems. Our previous studies indicate that NFI-C is required for tooth root development and bone formation, but the exact function of NFI-C in cartilage development remains unknown. In this study, *Nfic*$^{-/-}$ mice revealed decreased growth-plate lengths compared with WT. In particular, the width of the proliferating and hypertrophic zone in the growth plate was dramatically reduced in *Nfic*$^{-/-}$ mice compared with WT. However, NFI-C disruption has no influence on prenatal cartilage development. In addition, cell proliferation rates of *Nfic*$^{-/-}$ chondrocytes were decreased approximately 40% at 3 days and 70% at 5 days compared with WT, respectively. PCNA-positive cells were significantly diminished in the proliferating zone of *Nfic*$^{-/-}$ mice compared with WT. In contrast, chondrocyte apoptosis was increased in the hypertrophic zone of *Nfic*$^{-/-}$ mice compared with WT. *Nfic*$^{-/-}$ chondrocytes exhibited increased p21 expression but decreased cyclin D1 expression, strongly suggesting cell growth arrest due to the lack of *Nfic* activity. Further, *Nfic*$^{-/-}$ chondrocytes exhibited increased caspase-3 activation. These results indicate that NFI-C disruption results in decreased femur length caused by reduction in the width of the growth plate, decreased chondrocyte proliferation, and increased chondrocyte apoptosis.
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P232
Hyperbaric Oxygen Suppresses Wnt/B-Catenin Signalling in Degenerated Intervertebral **Disc Cells – *In vitro* and *In vivo* Study**
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Background: The activation of Wnt/β-catenin signalling promotes cellular senescence and induces matrix metalloproteinases (MMPs) expression in intervertebral disc (IVD). We investigated the effects of hyperbaric oxygen (HBO) on the Wnt/β-catenin signalling in degenerated human IVD cells and rabbit IVD models.
Methods: In vitro, nucleus pulposus cells (NPCs) were separated from the degenerated disc nucleus tissue by performing sequential enzymatic digestion. Control cells were maintained in 5% CO_2 / 95% air. The hyperoxic cells were exposed to 100% O_2 at 2.5 ATA in a hyperbaric chamber. The mRNA or protein levels of Wnt3a, β-catenin, aggrecan, type II collagen as well as MMP-3 and 9 were analyzed after HBO treatment. The translocation of β-catenin was detected by western blot after HBO treatment. In vivo, external axial loading in the rabbit IVD was used to induce the disc degeneration. After 14 days of mechanical loading, the custom-made external loading devices were removed. The HBO group was exposed to 100% oxygen at 2.5 ATM for 2 h daily. The control group was kept in housing cages with normal air. After 8 weeks, we investigated the effects of HBO on degenerated rabbit IVD by immunohistochemical assays.
Results: In vitro, the mRNA level of Wnt3a was down-regulated while that of aggrecan and type II collagen were up-regulated after HBO treatment. Western blot analysis showed decreased levels of translocated β-catenin in nucleus after HBO treatment. ELISA data showed HBO suppressed the expression of MMP-3 and MMP-9. In vivo, Safranin-O and TUNEL staining showed that mechanical loading induced IVD degeneration that increased proteoglycan (PG) lost and apoptosis of IVD. The levels of Wnt3a and β-catenin were down-regulated in degenerated disc tissue section after HBO treatment.
Conclusion: HBO treatment suppresses Wnt/β-catenin signalling and MMPs expression in degenerated human IVDs and rabbit model.
Disclosure: The authors declared no competing interests.

P233

HBO Treatment Suppresses Wnt/B-Catenin Signalling Pathway Activation Through LRP5 Catabolic Activity in Human OA Chondrocytes

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Background: Wnt/β-catenin signalling stimulates matrix catabolic activity in articular chondrocytes. However, little is known about the effects of hyperbaric oxygen (HBO) on the Wnt/β-catenin signalling in human osteoarthritic (OA) chondrocytes.
Methods: OA chondrocytes were separated from the OA cartilages by performing sequential enzymatic digestion. Control cells were maintained in 5% CO_2 / 95% air throughout the experiment. The hyperoxic cells were exposed to 100% O_2 for 25 min and then to 5% CO2/95% air for 5 min at 2.5 ATA in a hyperbaric chamber. The mRNA or protein levels of low-density-lipoprotein receptor-related protein 5 (LRP-5), β-catenin, aggrecan, type II collagen as well as NO, MMPs, and ADAMTSs were analyzed after HBO treatment. The translocation of β-catenin from cytosol to nucleus after HBO treatment was detected by western blot. To determine the β-catenin-Tcf/Lef transcriptional activity, we measured the activity of Tcf/Lef reporter gene Topflash (optimal Tcf-binding site) or Fopflash (mutated Tcf-binding site) in OA chondrocytes.
Results: The mRNA levels of TADAMTS4 and ADAMTS5 were down-regulated while that of aggrecan and type II collagen were up-regulated in OA chondrocytes after HBO treatment. ELISA data showed decreased proteins expression of NO, MMP3, MMP9, and MMP13 in OA chondrocytes after HBO treatment. The relative density ratio (phosho-protein/protein) for LRP5 was down-regulated after HBO treatment. Western blot analysis showed decreased levels of translocated β-catenin after HBO treatment. There was decreased TOP flash activity following HBO stimulation, whereas the FOP flash activity was not affected. HBO decreased Tcf-dependent transcription and suppressed the expression of MMP-9.
Conclusion: HBO treatment suppresses Wnt/β-catenin signalling, ADAMTSs, and MMPs expression through LRP5 catabolic activity in human OA chondrocytes.
Disclosure: The authors declared no competing interests.

P234

The Expression of RANKL, RANK and OPG in the Cartilage and Subchondral Bone in Patients with Osteoarthritis and Osteoporosis

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The objective of the study was to determine articular cartilage and subchondral bone expression of the bone regulating molecules receptor activator of nuclear factor κB ligand (RANKL), receptor activator of nuclear factor κB (RANK) and osteoprotegerin (OPG) in the hip osteoarthritis (OA) and hip osteoporosis (OP). Cartilage and subchondral bone samples were obtained from 40 patients undergoing total hip replacement surgery after end stage of osteartritis (15 patients) or femoral neck fracture (25 patients). Tissue sections were stained with Safranin O and graded. Immunohistochemical staining was then performed, and levels of RANKL, RANK and OPG expression were assessed using a semi-quantitative scoring system. In addition, levels of mRNA encoding for RANKL, RANK and OPG were determined by a real-time reverse transcription-polymerase chain reaction technique. We found that expression of RANKL protein, mRNA expression, and the ratio of RANKL: OPG mRNA was greater in the cartilage and subchondral bone in OA samples in comparison with OP samples ($P < 0.05$). Increased RANKL and staining in OA cartilage was predominantly in the peri-cellular region of the middle and deep zones as well as in the matrix of the superficial zone. OPG mRNA expression in OA samples was greater in the cartilage in comparison with subchondral bone ($P < 0.05$) while OPG mRNA expression in OP samples was greater in the subchondral bone in comparison with cartilage ($P < 0.05$). Cartilage and subchondral bone are in close proximity and soluble proteins produced in the cartilage are likely to move from one compartment to the other. Our finding of increased expression of RANKL in OA cartilage might explain the increase in bone turnover reported in the subchondral bone of OA patients.
Disclosure: The authors declared no competing interests.

P235

Histological Structure of the Proximal Epiphyseal Cartilage of Humerus after 60-Day Application of Tartrazine

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Background: The aim **was** to investigate structure of the proximal epiphyseal cartilage (EC) of humerus after 60-day tartrazine intake and to find possibility of correction of the state with mexidol.
Methods: The study involved 175 male rats with body weight of 200-210 grams. The first group comprised the intact animals (C group), the second and the third groups comprised the animals that received *per os* tartrazine in dosage of 750 and 1500 mg per kg of body weight for 60 days (T1 and T2), and the fourth and the fifth groups received IM tartrazine and mexidol in dosage of 50 mg per kg of body weight (T1M and T2M). Upon expiration of observation terms (the 3rd, the 10th, the 15th, the 24th, and the 45th day) the frontal sections of HE stained proximal epiphyses were put to morphometry of zones of EC using classification of V.G. Koveshnikov (2003).
Results: By the third day of observation in the samples taken from T1, width of EC was lower than that of controls by 8.59%, width of osteogenic zone was respectively lower by 9.92%, amount of primary spongiosa and quantity of cells on trabecules' surface were lower by 8.45% and 8.42%. In T2, the same values were lower by 11.52%, 11.91%, 9.84% and 9.49% as compared with the controls. From the 10th up to the 24th day, width of osteogenic zone for T1 was lower than that of C by 8.79%, 5.17%, and 5.32% and for T2 by 9.67%, 7.11%, and 5.33%. In the same period, osteoblast

count in osteogenic zone for T1 was lower than that of the controls by 10.52%, 7.62%, and 5.83% and or T2 those values were lower by 11.31%, 8.10%, and 6.42%, respectively. Total width of EC in both groups by the 45th day was lower in comparison with the controls. Administration of mexidol together with tartrazine resulted in structure optimisation of the EC as compared with T1 and T2. Values characteristic of bone formation activities of the EC in T1M were higher than in T1 in the period from the 3rd up to the 45th day of observation and in T2M – from the 15ht up to the 45th day of observation.
Conclusions: 60-day tartrazine intake results in inhibition of activities of EC of humerus. Degree and restoration rates directly depend on tartrazine dosage. Administration of mexidol in dosage of 50 mg per kg of body weight had a positive effect on restoration of epiphyseal cartilage of humerus in comparison with T1 and T2 groups.
Disclosure: The authors declared no competing interests.

P236
Signal Activation of Articular Chondrocytes by β-Endorphin
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β-endorphin is an agonist of the opioid receptors. It is a short peptide, resulting from processing of the precursor proopiomelanocortin (POMC). In the present study, the *in vitro* effect of β-endorphin stimulation on human articular chondrocytes was investigated. The primary cultures of articular chondrocytes from knee joint cartilage obtained at total knee replacement of patients with osteoarthritis were treated with β-endorphin at a concentration of 600 ng/ml for 0.5, 1, and 3 hours. Total cell lysates were collected for western blotting to analyse the signal molecules. Following stimulation of β-endorphin for 30 min protein Erk 1/2 showed significantly increased phosphorylation (1.5±0.206), which was inhibited by U0126, a specific inhibitor of MEK. Returned to basal level following 1 and 2 hours stimulation was noted. This effect was abolished by EGTA, an extracellular calcium ion chelator. Increased Erk 1/2 phosphorylation in the short term stimulation of β-endorphin may be beneficial for chondrocyte activity. The subsequent effect on chondrocytes will be further carried out.
Disclosure: The authors declared no competing interests.

CELL BIOLOGY: OSTEOCYTES

P216 (OP30)

P217 (OP31)

P218 (OP4)

P219
Effects of Monosodium Urate (MSU) Crystals on MLO-Y4 Cell Viability; is there a Role for Osteocytes in Bone Erosion in Gout?
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Background: Gout is the most common form of inflammatory arthritis. It is characterised by the deposition of monosodium urate (MSU) crystals within joints. MSU crystals are found within subchondral bone. Previous research has determined that patients with advanced gout have enhanced osteoclast-mediated bone resorption and impaired osteoblast-mediated bone formation, leading to focal bone erosion. The osteocyte has been shown to be a master regulator of bone remodelling. The aim of this study was to investigate the effects of MSU crystals on osteocyte-like cell viability.
Methods: MSU crystals were prepared by recrystallisation of uric acid. MLO-Y4 osteocyte-like cells were cultured on plastic (2D) or in 3 mg/ml type I collagen gels and cultured with 0.01-0.5 mg/ml MSU crystals or soluble uric acid for 24 hours (day 0). Cells were then washed and MSU crystals or uric acid completely removed. Cell viability was assessed 24 hours and 48 hours after the addition of MSU crystals using MTT and alamarBlue™ assays.
Results: In 2D cultures, 0.1-0.5 mg/ml MSU crystals significantly reduced MLO-Y4 cell viability by ~70% 24 hours after the addition of MSU crystals ($P<0.05$). In collagen gels, higher concentrations of MSU crystals (0.3-0.5 mg/ml) reduced MLO-Y4 cell viability by ~30-40% 24 hours after the addition of MSU crystals ($P<0.01$); whereas lower concentrations of MSU crystals (0.01-0.1 mg/ml) had no effect ($P>0.05$). However, 48 hours after the addition of MSU crystals, culture with the higher concentrations of MSU crystals (0.3-0.5 mg/ml) resulted in a 100% reduction of MLO-Y4 cell viability ($P<0.01$). The inhibitory effect on cell viability was specific to MSU crystals, as soluble uric acid did not reduce viability.
Conclusion: These results indicate that MSU crystals are toxic to osteocyte-like cells and that direct crystal-cell interactions are not necessarily required to reduce osteocyte viability. The interactions between MSU crystals and osteocytes may contribute to bone erosion in gout.
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P220
Isolation of Osteocytes from Human Trabecular Bone
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While several murine osteocyte-like cell lines are available and techniques for isolating osteocytes from mouse bone have been described, few models exist for studying human osteocytes *in vitro*. We have developed a method for isolating osteocytes from bone taken from patients undergoing knee arthroplasty. Trabecular bone was dissected and washed to remove marrow and subjected to sequential digestions in

collagenase/EDTA. Cells were harvested after each digest, plated on collagen coated wells and cultured over a 5 day time course. Osteocyte gene expression was analysed by RT-PCR and cell morphology examined by phalloidin staining. Cells harvested from digests 1 and 2 expressed low levels of the osteocyte markers *SOST* and *DMP1*, with increased levels observed in digests 3 and 4. The highest levels of these markers were observed in digests 5 and 6, with a 20-fold increase in *DMP1* and a 9-fold increase in *SOST* mRNA compared to digest 1. *FGF23* mRNA expression was absent in the early digests but was observed from digest 3 onwards, increasing up to 150-fold in expression in digest 6. The osteocyte markers *PHEX* and *MEPE* were also expressed in the isolated cells and were increased in the later digests. The cells isolated in digests 1 and 2 displayed a mixed morphology, with osteoblast-like cells and some dendritic osteocyte-like cells after 5 days of culture. Digests 3-6 contained many highly dendritic cells, which were initially observed after 2 days of culture and increased in number and dendricity after 5 days. Treatment of isolated cells from digests 3-6 with PTH or $1,25(OH)_2$vitaminD_3 for 24 hours resulted in the downregulation of *SOST* and upregulation of *FGF23* mRNA levels, respectively, similar to osteocytes *in vivo*. In conclusion, we have developed a reproducible method of isolating osteocytes from human bone. Such cells will be invaluable for furthering osteocyte research.

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P221

Cre-mediated Recombination Occurs in Various Organs in a Dmp1-Cre Reporter System

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Osteocytes are key players in regulating bone mass maintenance and are therefore a major research focus in the field of bone and mineral biology. Analysing osteocyte function often requires *in vivo* investigations. Therefore, promoter fragments of osteocyte-specific genes i.e. SOST or the 8kb and 10kb fragments of the Dentin-matrix-protein 1 (Dmp1) were used to overexpress genes of interest or Cre-recombinase for conditional deletion studies using the Cre-loxP system. While these tools have been very useful to investigate osteocyte biology, evidence emerged suggesting that these promoters are not osteocyte-specific, which is very important for the data interpretation. To further investigate the specificity of a supposedly osteocyte-specific Cre-loxP-system, we crossed the 8kb-Dmp1-Cre mice with Ai9 reporter mice in which a loxP-flanked STOP cassette prevents the expression of tdTomato (*Dmp1-Cre^{+};Ai9$^{T/wt}$*). At 8-weeks of age, male mice were sacrificed and various organs were harvested. Tibiae were decalcified and all tissues were paraffin-embedded, followed by tomato detection using immunohistochemistry. In bone, tomato was strongly expressed in all osteocytes and osteoblasts covering endocortical and trabecular surfaces, while no expression was found in articular cartilage of *Dmp1-Cre^{+};Ai9$^{T/wt}$* mice compared to *Dmp1-Cre^{-};Ai9$^{T/wt}$* and *Dmp1-Cre^{+};Ai9$^{wt/wt}$* control animals. Furthermore, we detected tomato expression in muscle, brain, testis, and in vessels in the heart, spleen, lung, and intestine. We did not observe tomato expression in the kidney, liver, fat, or skin. These results indicate that in the *8kb-Dmp1-Cre;Ai9* reporter system, Cre-mediated recombination occurring during mouse development and growth is not restricted to osteocytes, but also takes place in other osteolineage cells and in several organs known to be functionally involved in the regulation of bone homeostasis. Our findings therefore suggest that despite the great usefulness of conditional gene deletion systems, the expression pattern of the gene of interest should be determined carefully and the findings need to be interpreted accordingly.

Disclosure: The authors declared no competing interests.

P222

Inflammatory Cytokines Affect the Production of Osteocyte-Related Signalling Molecules by Human Bone Cells Cultured in Close Contact with their Native Matrix

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Bone remodelling is disturbed in rheumatoid arthritis (RA) possibly because of elevated levels of circulating inflammatory cytokines. Osteocyte signalling plays a vital role in bone remodelling by affecting bone formation and/or bone resorption. Therefore, we aimed to investigate the effect of RA-serum containing inflammatory cytokines and exogenous recombinant inflammatory cytokines on human osteocyte signalling. Human trabecular bone chips were denuded by 2h collagenase treatment. Bone chips were cultured with or without 10% active RA-serum, and with or without recombinant IL-1β, IL-6, IL-17, TNFα (concentration: 10 ng/ml), or a cytokine cocktail (IL-1β, IL-6, TNFα) for 7 days. Live-dead staining was performed to assess cell viability. Gene expression of cytokines and osteocyte signalling proteins was analysed by qPCR. Only few cells were observed on the surface of bone chips at day 0, while approximately 80% of the surface was covered by cells after 7 days. Cells in or on the bone chips did express the osteocyte markers sclerostin, FGF23, DKK1, MEPE, IL-1β, and TNFα at day 0 and 7. Treatment with RA-serum, IL-1β, or TNFα enhanced gene expression of IL-1β (8-15–fold) and TNFα (2-3–fold). Treatment with IL-1β or TNFα, but not RA-serum, also enhanced gene

expression of IL-6 (25-32–fold) and IL-8 (24-58–fold). The stimulatory effect of the cytokine cocktail on gene expression of IL-1β, IL-6, and IL-8 was significantly higher (80-120–fold) than the effect of the individual cytokines. IL-1β, TNFα, and the cytokine cocktail enhanced FGF23 expression (2-4–fold). Sclerostin expression was only enhanced by IL-1β (5-fold). RA-serum increased both sclerostin expression (2.5-fold), and DKK1 expression (2-fold). In conclusion, RA-serum and exogenous recombinant cytokines changed osteocyte signalling in cultured human denuded bone chips containing cells that express osteocyte markers, which suggests that osteocytes could provide a new target to prevent bone loss in inflammatory diseases.
Disclosure: The authors declared no competing interests.

P223
Variation in Osteocyte Lacunar Size and Shape in Rapidly Forming Cortical Bone Due to Mechanical Loading
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Background: Cortical bone is a dynamic, living tissue that has a hierarchically organised microstructure which is able to repair itself and adapt to dynamic mechanical loading. Bone's adaptation to changes in its mechanical environment is controlled at the cellular level. Osteocytes, the primary mechanosensory cells, reside in osteocyte lacunae. Variations in the dimensions of osteocyte lacunae have been reported; however, the role of these variations in bone's adaptive response is unknown. The aim of this study was to explore potential variations in osteocyte lacunar size and shape in bone following mechanical loading.
Methods: A fibula of a C57Bl/6J male mouse was subjected to 2-week *in-vivo* mechanical loading utilising the noninvasive murine tibia loading model (three times per week, 16.5N, 40 pulses, 10s interval; ethical approval had been obtained). The contralateral fibula served as internal control. Both fibula were scanned nondestructively using a conventional desktop micro-CT (Skyscan 1172) at 5 μm resolution. Subsequently, 1.4 mm of the proximal part of the loaded fibula was rescanned at a nominal resolution of 700 nanometer. After reconstruction and segmentation channels and lacunae were visualized and quantified.
Results: In the loaded fibula, substantial periosteal bone formation had occurred at the periosteal surface of the proximal region; this was not seen in the contralateral fibula. The new bone showed a disorganised woven-bone-like pattern. The newly formed bone exhibited higher lacunar density and variations in shape and size. Mean lacunar volume (593 μm^3) at the border of pre-existing cortical bone and newly formed bone was nearly twice as high as in the midcortical regions (306 μm^3).
Discussion: Osteocyte lacunae formed in rapidly forming bone have a morphology that differs from spatially closely-related osteocytes. NanoCT protocols may offer 3D insight into bone microstructure and its regulation by mechanical loading.
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GENETICS

P237 (OP36)

P238 (OP37)

P239
***PLS3* Gene Variation in Childhood Fractures and Primary Osteoporosis**
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Mutations in the gene *PLS3* have recently been found as being a cause of early-onset primary osteoporosis. This type of osteoporosis affects males more often and more severely than females, because *PLS3* is located on the X-chromosome. The encoded protein Plastin3 functions as an actin bundling protein and is abundantly expressed in solid tissue. *PLS3* is highly expressed in osteocytic dendrites and is likely to have a role in skeletal mechanosensing. The aim of this study is to explore the question of how prevalent *PLS3* mutations are in childhood-onset primary osteoporosis. Altogether 32 patients with childhood-onset primary osteoporosis, without a molecular diagnosis, were included. The diagnosis of primary osteoporosis was based on low BMD with a history of recurrent, low impact, peripheral fractures or vertebral compression fractures and exclusion of secondary osteoporosis. Using PCR and Sanger sequencing techniques, all coding exons and exon-intron boundaries of the *PLS3* gene were sequenced for all the 32 patients. A previously not described nonsense mutation in exon 8, c.766C>T; p.Arg256X, was found in a presently 28-year-old male, with a history of multiple vertebral compression fractures since early childhood and a markedly low BMD. He also has slightly blue sclerae and joint hyperlaxity. The *PLS3* mutation was inherited from his mother, who has osteopenia and joint hyperlaxity. Based on cDNA analysis the mutation results in synthesis of a truncated protein as mutated RNA escapes nonsense-mediated RNA decay. Several *PLS3* variants were found in the remaining patients but none of these variants were regarded as disease-causing. In conclusion, *PLS3* mutations explain some cases of early-onset osteoporosis but our results do

not support first-line screening of *PLS3* in patients with childhood-onset primary osteoporosis. The pathogenetic mechanisms through which *PLS3* mutations lead to the phenotypic manifestations need to be explored in future studies.
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P240
Modulation of Osteoblast-Related Genes in Fibroblasts by Homocysteine and Bisphosphonates
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The aim of the present study was to examine effects of homocysteine (Hcys), and bisphosphonates (BPs) on fibroblast gene expression for exploring its potential utility as surrogate for monitoring bone matrix quality. NIH-3T3 mouse fibroblast cells were cultured for six days in the presence/absence of one of the most widely used bisphosphonates in osteoporosis management (alendronate, ibandronate, risedronate; 1μM), with/without 4 mM Hcys. Following cell lysis and total RNA extraction, reverse transcriptase real-time PCR was performed on extracts of three independent experiments, data of which were evaluated using Student´s paired *t*-test. We identified a number of genes affecting bone quality and implicated in osteoporosis whose expression was statistically significantly modulated by Hcys and BPs in fibroblasts. Effects included down-regulation of *Il6* by Hcys by one third ($p<0.05$) relative to untreated control cell cultures as well as up-regulation of lysyl oxidase (*Lox*) and periostin (*Postn*) in response to Hcys in combination with either alendronate or ibandronate by 1.5-fold to 2.2-fold ($p<0.05$). Moreover, when cells were incubated with Hcys plus risedronate, a similar effect was observed on *Igf1* (insulin-like growth factor 1) expression, which was increased 2.4-fold ($p<0.05$) compared with untreated control. The results show that fibroblasts are responsive to stimuli that osteoblasts are exposed to in osteoporosis. Interestingly, in osteoblasts the opposite effects of Hcys alone on *Il6* expression (up-regulation) and *Lox* expression (down-regulation) to the ones found herein were described previously. Moreover, elevated serum POSTN levels and decreased serum IGF-1 concentrations have been linked to increased fracture risk and osteoporosis, respectively. Since fibroblasts are responsive to both Hcys and bisphosphonates, skin organic matrix quality might be reflective of bone organic matrix quality both in disease and under treatment.
Disclosure: The authors declared no competing interests.

P241
Connecting to Bone Data from the International Knockout Mouse Consortium (IKMC) and Other Databases
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The IKMC is analysing multiple phenotypes in adult KO mice for all 20,000+ protein-coding genes. Data are available at the International Mouse Phenotyping Consortium (IMPC) website and KO mice generated can be readily obtained. KO of approximately 30% of genes results in lethality. Body BMD values for males and females provide an initial characterisation of skeletal KO phenotypes. High-resolution radiographs provide dysmorphology information, such as digit, spine and craniofacial abnormalities. The MGI Gene Expression Database (GXD) provides RNA expression profiles for multiple tissues, including bone. The JAX *Cre* Repository contains over 300 *Cre* tool mouse strains. Mutant mice available are listed on website of the IMPC, MGI and Mutant Mouse Regional Resource Centers. JAX, Welcome Trust Sanger Institute, MRC Harwell, and German Mouse Clinic websites, among others, often have more comprehensive data than the IMPC website. These resources provide valuable information to the bone community. For any candidate gene of interest, IMPC data showing lethality, the lack of a bone phenotype, the presence of a bone phenotype, and/or the presence of non-skeletal phenotypes can guide decisions for individual laboratories. The IMPC database currently provides complete phenotype data for 492 KO mouse genes with phenotyping underway for an additional 767 KOs. The number of genes examined is anticipated to increase rapidly during the next few years. Efforts are underway with BoneKEy to develop an annotated web database focused on IMPC bone data. Two examples are informative. Confirming published data on *Lrrk1* osteopetrotic KO mice, body BMD is elevated in IMPC KO mice. A separate KO mouse, available from JAX and involving a different KO strategy, shows neonatal lethality. Confirming published data on *Wnt16* KO mice suffering spontaneous fractures from reduced cortical bone mass, IMPC KO mice also exhibit spontaneous fractures. *Wnt16* expression is restricted to bone, testes and the vasculature.
Disclosure: The authors declared no competing interests.

P242
Resequencing of WNT16 in Extreme BMD Groups from the BARCOS Cohort: Detection and Association Analysis of Common and Rare Variants
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Osteoporosis is a complex disease determined by both genetic and environmental factors. Genetic influence is polygenic, and this defect is caused by the additive effect of many susceptibility genes, as shown by several GWAs including

the last metaanalysis by Estrada *et al.* (2012). In this study, 56 loci were found associated with BMD, 14 of which were also associated with osteoporotic fracture. Several of these genes belong to the Wnt signaling pathway, including *WNT16* (rs380187). To better understand the role of *WNT16* in BMD determination and fracture susceptibility, we aimed to explore the allelic architecture of *WNT16* by resequencing all coding exons in two extreme BMD groups from the BARCOS cohort: 55 women with the highest BMD (*HBM*) and 53 with the lowest BMD (*LBM*). Once these variants were determined, the most promising were genotyped in the complete BARCOS cohort. Association was tested by ANCOVA, adjusting by years since menopause. We found 17 SNVs, all previously described. Five of them were observed in only one or two samples. Although none of them presented significant differences between the extreme groups, a trend was observed for 4 of them. These 4 SNPs and the 5 rare variants were genotyped in n=1625 women form BARCOS. Nominal significant results were obtained for SNVs rs2707466, rs142005327 and rs2908004. SNP rs2707466 and rs2908004 are missense variants (p.T253I/p.T263I and p.G72R/p.G82R, respectively) and in our cohort they are in strong linkage disequilibrium with rs380187 (the "GWAs hit"). rs142005327 is an intronic 2-bp insertion, previously found associated to BMD by Hendrickx et al. (2014). One of the rare variants was found in only one HBM woman of the BARCOS cohort. It is an intronic change located in a putative transcriptional regulation site.
This study adds evidences on the role of WNT16 in bone biology.
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P243

Genetic Screening of WNT5B in Patients with Monogenic Sclerosing Bone Disorders and Healthy Men with an Extreme Bone Mineral Density

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The past decade, genome wide association studies (GWAS) have been performed to reveal the links between genomic variation and the occurrence of osteoporosis. These studies revealed the *WNT5B* gene, being associated with bone mineral density (BMD) at the level of the femoral neck and the lumbar spine with genome-wide significance ($p< 5x10^{-8}$). This finding made *WNT5B* interesting for further genetic studies to verify the influence of common and rare genetic variation in this gene in patients with high BMD disorders and on the BMD of healthy individuals. First, a mutation screening was performed in a population of patients with monogenic sclerosing bone disorders (n=43), which all tested negative for mutations in the known causative genes (*SOST*, *LRP5* and *LRP4*). All coding exons of *WNT5B* and their intron-exon boundaries were analysed by using Sanger sequencing. Here, *WNT5B* showed no disease-causing mutations. Moreover, re-sequencing of *WNT5B* was performed on healthy men of the Odense Androgen Study (OAS). Here, based on their extreme high or low BMD values, two cohorts of 63 subjects each were selected. Again, all coding exons and their intron-exon boundaries were screened for rare and common variation using Sanger sequencing. As a result, no coding variation could be detected in the *WNT5B* gene. Five intronic variants were detected to be varying across the cohorts, but showed no significant difference in genotype frequencies between the lower and higher BMD cohort. Despite the results from GWAS in the past, we were not able to replicate or further verify an important role for *WNT5B* in the genetic determination of BMD.
Disclosure: The authors declared no competing interests.

P244

Search and Functional Evaluation of Rare Variants in *RSPO3* Gene

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R-spondin 3 is a member of the RSPO family of secreted Wnt-signalling agonists, an important pathway in the regulation of bone metabolism. Furthermore, genetic variation in RSPO3 is previously reported to be associated with BMD. Therefore, the aim of this study was to confirm the previously reported association and to identify a possible causative variant by screening the exons of RSPO3 using Sanger sequencing in two subpopulations of the Odense Androgen Study (OAS). The two subpopulations contain the 64 individuals with the lowest and highest BMD-values (*t*-score <-1.38 and *t*-score >1,54). We identified 4 known common intronic variants, however, genotype frequencies didn't differ significantly between the high and the low BMD cohort. In addition, we identified one rare coding variant (rs140821794) located in exon 1. The variant was found in one individual (T-score=-1,515) and according to the prediction program SIFT, the variant has a deleterious effect on the protein function while Polyphen2 predicts that is benign. Consequently, we tested the effect of the variant on canonical Wnt-signalling with an *in vitro* reporter assay in HEK293 and Saos-2 cells. Although we were able to show cell-type dependent differences in the activation of the pathway by RSPO3, we didn't find a significant difference in the effect of the WT or mutated RSPO3 on Wnt-signalling activity. In conclusion, we were not able to replicate the previously reported associations of common genetic variation in RSPO3 with BMD in our population. Although we did identify a possible interesting rare variant, we were not able to show an effect of the variant on the regulation of canonical Wnt signalling using functional studies. This can be due to the fact that the effect is too small to detect with our assay. Therefore, more studies are needed to elucidate the role of RSPO3 in maintaining bone mass.
Disclosure: The authors declared no competing interests.

P245

Identification of miRNAs Associated with Osteoblastic Differentiation

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At 21-25 nucleotides long, MicroRNAs (miRNA) are non-coding, regulators of gene expression at the posttranscriptional level through degradation and/or inhibition of mRNA translation. miRNAs have been associated with various human diseases, although its precise role in metabolic bone diseases such as osteoporosis is unclear. The aim of this study is to investigate the involvement of miRNA at different stages of human bone development. We investigated miRNA expression in three human osteosarcoma cell lines representing different stages of osteoblast differentiation, MG-63 the least differentiated, TE-85 intermediate and SaOS-2 the most mature. In the other study, we found that SaOS-2 expressed the highest level of sclerostin and alkaline phosphatase followed by TE85 and MG63. This result confirms that SaOS-2 is the most mature while MG63 is the least differentiated. All cell lines were cultured in DMEM + 10% FCS. Total RNA was extracted from confluent cell culture. More than 500 miRNAs were differentially expressed, those with the largest difference in expression were hsa-miR-935, hsa-miR-143-3p, hsa-miR-145-5p, hsa-miR-155-5p, hsa-miR-3200-3p, hsa-miR-584-5p, hsa-miR-486-3p, hsa-miR-767-5p and hsa-miR-105-5p. miR-935 was expressed highest in TE85 and lowest in SaOS-2 ($p<0.05$). Expression of miR-143-3p and miR-155-5p was significantly higher in TE85 and SaOS-2 cells compared to MG63 ($p<0.05$). However, MG63 showed highest expression of miR-155-5p, miR-3200-3p and miR-584-5p compared with TE85 and SaOs-2 ($p<0.05$). The most striking observation to emerge from the data comparison was the expression of miR-155-5p in MG63 was 2842-fold greater than SaoS-2 and 1467-fold greater in TE85 than SaOS-2. Meanwhile, miR-486-3p, miR-767-5p and miR-105-5p were highest in SaOS-2 compared with MG63 and TE85 ($p<0.05$). Our data shows that different stages of osteoblast development are characterised by different sets of highly expressed microRNAs and suggests that miRNAs could be potential biomarkers in bone development and may provide the basis of new therapeutic approaches to prevent bone loss.

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P246

Mutations in the *LRP4* and *LRP5* Genes are Associated with Bone Mineral Density in Maltese Postmenopausal Women

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Background: Osteoporosis is a multifactorial skeletal disease characterised by low bone mass leading to increased fracture risk. Members of the low-density lipoprotein receptor-related protein (*LRP*) gene family play a role in osteoblastogenesis through the Wingless (Wnt)/βeta-catenin pathway. *LRP4* controls the actions of sclerostin, a Wnt inhibitor, whereas *LRP5* promotes bone formation. The aim was to evaluate the effect of four non-synonymous coding polymorphisms in relation to bone mineral density (BMD) and low-trauma fractures in Maltese postmenopausal women. Genotyped variants were the *LRP4* rs2306033 (C>T) and rs6485702 (G>A) variants, and the *LRP5* rs4988321 (G>A) and rs3736228 (C>T) variants.
Methods: Research subjects were 1045 women aged 40 to 79 years, subdivided in three BMD groups without a fracture history: normal, osteopenic or osteoporotic. Women with a fracture history were classified as cases. Genotyping was performed by polymerase chain reaction and restriction fragment length polymorphism, or by real-time PCR. Odds ratios were computed using logistic regression analysis adjusted for age and clinical risk factors.
Results: Homozygosity for the rs6485702 G allele was found associated with low BMD at the lumbar spine, LS (P=0.01) relative to research subjects with a normal BMD, whereas heterozygotes for this allele had a low BMD at the femoral neck, FN (P=0.04). Women carrying one or two copies of the rs3736228 T allele and one copy of the rs4988321 A allele had a lower BMD at the LS and FN (P<0.05). The rs6485702 and rs3736228 variants were associated with increased fracture risk, however this was not independent of BMD. Interactions were observed between these three variants at the LS (P<0.01). Women carrying the *LRP4* C-G haplotype and *LRP5* A-T haplotype had lower BMD measurements at the LS and FN (P<0.05).
Conclusion: The *LRP4* rs6485702 variant and the two *LRP5* variants play a role in BMD regulation in Maltese postmenopausal women.
Disclosure: The authors declared no competing interests.

P247

Polymorphisms in Neuropeptides Genes and Bone Mineral Density in Postmenopausal Korean Women

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Background: The purpose of this study was to investigate the association between single nucleotide polymorphisms in neuropeptide genes and bone mineral density (BMD) in postmenopausal Korean women.

Methods: The 20 polymorphisms in the neuromedin (NMU) gene, NMU receptor 2 (NMU2R) gene, cocaine- and amphetamine-regulated transcript (CART) gene, neuropeptide Y (NPY) gene, NPY receptor 2 (NPY2R) gene, neuronal nitric oxide synthase (NOSI) gene, and melanocortin 4 receptor (MC4R) gene were analysed in 482 postmenopausal Korean women. Serum levels of bone turnover makers, leptin, soluble leptin receptor (sLR), osteoprotegerin (OPG), and soluble receptor activator of the nuclear factor-κB ligand (sRANKL) were measured and the BMD at the lumbar spine and femoral neck were also examined. The Institutional Review Board of our institution approved the study protocol.
Results: The CART rs2239670 was related to BMD of the lumbar spine, and the AG genotype had the highest BMD. Osteoporosis of the lumbar spine was more frequently observed in the GG genotype of the NPY rs17149106 and the CC genotype of the NPY rs16123 and less frequently observed in the TT-TT genotype identified by a combined polymorphism in the NPY2R gene, compared with the corresponding genotype. The AA genotype of the NOSI rs1279104 was found to have a 3.68-times higher frequency of osteoporosis at the femoral neck than the GG genotype. The adjusted serum levels of bone turnover markers, leptin, sLR, FLI, OPG, sRANKL, or sRANKL×1,000/OPG were not associated with the single polymorphisms measured in neuropeptide genes.
Conclusion: Our results suggest that the CART rs2239670 may be one of the genetic factors affecting lumbar spine BMD in postmenopausal Korean women, and that the NPY rs17149106, and rs16123, NOSI rs1279104, and combined polymorphism (rs2880415, rs6857715) in the NPY2R gene may be useful in identifying women at risk of osteoporosis.
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P248

ACE Gene I/D Polymorphism in Han Chinese Patients with Osteoarthritis

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Osteoarthritis(OA) is a degenerative joint disorder resulting in destruction of articular cartilage, osteophyte formation, and subchondral bone sclerosis. Angiotensin converting enzyme (ACE) plays an prominent role to promote inflammation and has been particularly related to rheumatic and autoimmune diseases. It is a metalloenzyme converts angiotensin I to a potent vasoconstrictor angiotensin II and will inactivate bradykinin which is a vasodilator may implicates immune-related disease including osteoarthritis. The aim of the current study was to examine the influence of ACE gene insertion/deletion (I/D) variations on the risk of osteoarthritis. A total of 460 patients with OA and 400 healthy subjects, both of them from healthy evaluation centres were included in the study. The definition of OA is readers evaluated Kellgren-Lawrence grade ≥2 by using X-rays judgment. DNA was extracted from a peripheral blood sample and was amplified by PCR using I and D allele-specific primers. PCR products were assessed with via UV visualisation by being exposed to 1.5% agarose gels. A significant difference between patients and controls was found that in the frequencies of ACE I/D alleles, OA patients had a higher presence of D allele (OR=1.8 , 95% CI=1.23–2.27, $p<0.001$) and the DD genotype (OR=2.03, 95% CI=1.24–3.03, $p<0.001$). Our results revealed that ACE Gene I/D polymorphism may be associated with osteoarthritis, ACE Gene polymorphism could be used as genetic markers in osteoarthritis in Han Chinese populations.
Disclosure: The authors declared no competing interests.

P249

Association of Polymorphism of RAGE Gene with Knee Osteoarthritis in the Taiwanese Population

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Osteoarthritis (OA) is a degenerative joint disorder resulting in destruction of articular cartilage, osteophyte formation, and subchondral bone sclerosis. The S100 family and receptor for advanced glycation end products (RAGE) participate in regulating inflammation, even in the production of matrix metalloproteinases (MMPs). MMP-1 degrades cartilage, which may result in OA development. Moreover, polymorphisms in RAGE, S100A8, and MMP-1 have a marked effect on ligand binding and transcription regulating. In this study, we investigated the potential genetic contribution of the RAGE, S100A8, and MMP-1 genes to OA. We performed a matched case-control association study and genotyped OA patients and healthy controls, who were analysed by polymerase chain reaction-restriction fragment length polymorphism assays. A total of 314 patients were diagnosed with knee OA and underwent total knee replacement. The control group included 268 individuals who had standard X-rays of the knee joints to confirm $K/L < 2$ and were matched by age and gender. Single-nucleotide polymorphisms in RAGE (557G/A), S100A8 (rs3795391A/G), and MMP-1 (-519A/G) were evaluated. S100A8 rs3795391A/G and MMP-1 -519A/G showed no significant difference between OA patients and healthy controls. RAGE 557G/A showed a significant association between OA patients and healthy controls ($P < 0.05$, respectively). Our results suggest that the investigated polymorphism in the RAGE gene play a role in OA in the Taiwanese population.
Disclosure: The authors declared no competing interests.

P250

Association of Polymorphism of Matrix Metalloproteinase-1(MMP-1) Gene with Knee Osteoarthritis in the Han Chinese Population

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Osteoarthritis (OA) is a degenerative joint disorder resulting in destruction of articular cartilage, osteophyte formation, and subchondral bone sclerosis. One of the mechanisms of cartilage degradation in osteoarthritis is enzymatic proteolysis of the extracellular matrix by metalloproteinases. The aim

of this study was to investigate whether polymorphisms in the promoter of matrix metalloproteinase-1 (MMP-1), MMP-3 and MMP-9 genes were associated with susceptibility to knee osteoarthritis in the Han Chinese population. We performed a case control study. Three hundred cases comprised patients with a radiologic scoring (Kellgren-Lawrence score) ≥2 and 288 controls with a radiologic scoring (K-L score) <2. DNA was extracted from a peripheral blood sample, and the MMPs genotypes were determined by PCR-RFLP. Functional assay were performed using enzyme-linked immunosorbant assay (ELISA). The association between OA and control groups at -1,607 1G/2G (rs1799750) of MMP-1 gene polymorphism showed significant difference ($p < 0.05$) in both populations. However, no associations were found between OA and MMP-3 -1,306 C/T (rs243865), and MMP-9 -1,562 C/T (rs3918242). There were no significant differences in serum MMP1 levels between various genotypes of 1G/2G ($p > 0.05$). The present study showed significant association at MMP-1 promoter polymorphism (-1,607 1G/2G) and susceptibility to knee OA in the the Han Chinese population.
Disclosure: The authors declared no competing interests.

P251
Association of the Tissue Inhibitor of Metalloproteinases-3 (TIMP-3) Gene Polymorphism with Knee Osteoarthritis in Taiwan population
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Osteoarthritis (OA) is characterised by the progressive failure of the extracellular cartilage matrix, which leads to the destruction of articular cartilage and pathologic changes in the joints. Studies have investigated the main cause of degrading cartilage is governed mainly by a balance between the degradation of matrix metalloproteinases (MMPs) and their tissue inhibitors of metalloproteinases (TIMPs). The aim of this study was to identify whether functional polymorphisms in the promoter region of the TIMP-3 G/A (rs715572) and MMP-3 5A/6A (rs3025058) genes were associated with knee osteoarthritis in Taiwan population. The present study is based on 210 Taiwanese OA patients as case group with a radiologic scoring (Kellgren-Lawrence score) ≥2 and 195 healthy subjects as control group with a radiologic scoring (K-L score) 0 and 1.Peripheral blood sample(5 ml) was collected from patient and healthy group. DNA was investigated by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). In our study, we found the association that TIMP-3 G/A (rs715572) gene polymorphism was significant difference between case and control group ($P = 0.032$). Otherwise, no association of MMP-3 5A/6A (rs3025058) gene polymorphism was found between case and control group ($P > 0.05$). These results have demonstrated a significant association between the polymorphism of TIMP-3 G/A gene with the susceptibility of OA in the Taiwanese population.
Disclosure: The authors declared no competing interests.

P252
Association of *MTHFR* C677T Polymorphism with Knee Osteoarthritis in Han Chinese Population
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Latest findings interpreted that not only degenerate, but also inflammatory are considered to involve in the development and progression of osteoarthritis (OA). Methylenetetrahydrofolate reductase (MTHFR) gene has been correlated with inflammation process. The function polymorphism C677T (rs1801133) mutation of MTHFR may lead to hyperhomocysteinemia so that enhances productions of several pro-inflammatory cytokines, such as IL6. In the light of these findings, we decided to survey if any relationship between MTHFR gene C677T mutation in Han Chinese population with knee osteoarthritis. We performed a case control study. 300 cases comprised patients with a radiologic scoring (Kellgren-Lawrence score) ≥2 and 500 controls with a radiologic scoring (K-L score) <2, both of them form healthy evaluation centres in Tri-Service General Hospital in Taipei, Taiwan. DNA was extracted from a peripheral blood sample, and the MTHFR gene C677T genotypes were determined by PCR-RFLP. Functional assay were performed using enzyme-linked immunosorbant assay (ELISA). Our results revealed that the MTHFR C677T polymorphism might increase the risk of OA (T allele vs. C allele: OR=1.78, 95% CI=1.01~3.14, $p<0.05$) after adjusted confounders, such as age, BMI, habitual physical activity. There were no significant in serum IL6 levels between various genotypes of C/T ($p > 0.05$). These data suggest that MTHFR C677T polymorphism may be associated with osteoarthritis, MTHFR gene could be used as genetic markers in osteoarthritis in Han Chinese populations.
Disclosure: The authors declared no competing interests.

P253
Genetic Risk Factors for Osteoporosis in Ukrainian Population
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Determination of molecular genetic causes of osteoporosis is an actual problem. There are several approaches to assess the contribution of a candidate gene in the pathogenesis of osteoporosis. One of them consists in determining the correlation between allelic polymorphism and candidate factors which cause the disease, which in comparison estimates allele frequencies of candidate genes in osteoporosis patients with individuals not having the disease and preserving normal bone mineral density. The aim of the work was to determine the alleles frequency of genes - regulators of bone metabolism in patients with osteoporosis in Ukrainian population, and to assess the contribution of different polymorphisms in the risk of developing the disease. DNA extraction was performed using the phenol-chloroform method from whole blood. Using PCR followed by restriction digestion and visualisation of the reaction products in polyacrylamide gel have been studied 180 patients with osteoporosis and 160 healthy people of the same age. We have found association

of polymorphism 60890 A/G of vitamin D receptor gene (OR= 3,2 (CI 95% 2,2-4.6)) and -234 T/G polymorphism of collagen type 1 gene (OR= 2,8 (CI 95% 2,1-4.1)) with the risk of osteoporosis developing. We have not found association of polymorphism -764 T/G of oestrogen receptor gene (OR= 1,2 (CI 95% 0,6-2,3)). Knowing association between pathogenic alleles, candidate genes and osteoporosis in Ukrainian population will allow to use genetic testing to identify predisposition to the disease. The results of this study are important for a more rational organisation of the prevention and treatment of the illness in the early stages of disease development.
Disclosure: The authors declared no competing interests.

MUSCLE, PHYSICAL ACTIVITY AND BONE

P254
Changes in Markers of Bone Turnover and Sclerostin during Arduous Military Training

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Background: Increased mineralisation and periosteal expansion of the tibia following short-term military training suggests changes in bone turnover, favouring formation. Sclerostin, which has anti-anabolic effects and is inhibited by mechanical loading, has recently been identified as a key regulatory marker in mechanotransduction of bone. The aim of this study was to examine the effects of 10 weeks arduous military training on markers of bone turnover and sclerostin.
Methods: Male Army recruits (*n*=82) undertaking initial Infantry training volunteered to participate. Early morning blood samples (0500 – 0545h) were collected at Baseline, Mid and End (weeks 1, 5 and 10) of training following an overnight fast and analysed for markers of bone formation (P1NP and OC), resorption (βCTX), sclerostin, regulatory markers of calcium metabolism (intact PTH, albumin adjusted Ca and PO_4) and vitamin D (total 25(OH)D). Data were analysed using repeated measured ANOVA (SPSS v19); statistical significance was set *a-priori* at $P<0.05$.
Results: P1NP increased from Baseline to Mid, returning to Baseline values at End (112.5 ± 67.6, 126.3 ± 69.2, 111.2 ± 62.3 μg/l). OC decreased from Baseline at Mid and End (50.2 ± 28.3, 43.9 ± 22.2, 40.0 ± 20.5 ng/ml). βCTX decreased from Baseline to Mid, returning to Baseline values at End (1.08 ± 0.49, 0.90 ± 0.49, 1.03 ± 0.45 ng/ml). Sclerostin decreased from Baseline at Mid returning to Baseline values at End (42.45 ± 16.9, 37.1 ± 13.4, 40.1 ± 13.1 pmol/l). 25(OH)D decreased from Baseline to End (72.1 ± 21.3, 41.8 ± 15.6 nmol/l) and increases in Ca and PO_4 were observed. iPTH remained unchanged.
Conclusions: These findings suggest an early, transient and orchestrated change of bone markers to increase bone density and alter bone structure in response to mechanical loading. These findings strongly support *in vivo* a role of sclerostin in mechanotransduction.
Disclosure: The authors declared no competing interests. This work was sponsored by the UK Ministry of Defence (Army).

P255
Physical Activity and Incidence of Vertebral Fracture, Disc Height Narrowing, and Facet Joint Osteoarthritis in Women and Men: the Framingham Study

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The effect of physical activity on spine health is poorly understood, particularly with respect to individual effects on bone, disc, and joint. We conducted a longitudinal study to determine the association between physical activity and incidence of VF, disc height narrowing (DHN), and facet joint osteoarthritis (OA) in women and men. Participants included 624 women and 512 men (mean age 62yr; range, 40-85yr) of the Framingham Study with baseline and 6-yr follow-up CT scans. VF, DHN, and OA from T4-L4 were scored as: 0=normal, 1=mild, 2=moderate, or 3=severe. Incidence was defined as any level scored ≤1 at baseline and ≥2 at follow-up. Physical activity was assessed using the Framingham Physical Activity Index (PAI). Robust Poisson regression was used to compute relative risk (RR) and associated 95% confidence intervals (CIs) quantifying the estimated association between PAI and incidence of VF, DHN, and OA, adjusted for age, height, weight, and smoking. Incidence of VF was 6% in women and men; DHN 40% women, 33% men; OA 35% women, 25% men. Mean PAI (range 26-78) ±SD was 37±6 in women and 38±7 in men. In women, VF incidence appeared to increase with higher PAI (trend, $p=0.08$), but there was no association in men (TABLE). There was a suggestion of a trend in increasing incidence of DHN with increasing PAI in men (trend, $p=0.05$) but not women (trend, $p=0.41$). In contrast, incidence of OA declined with increasing PAI in men (trend, $p=0.03$) but there was no association in women (trend, $p=0.63$). We found that increased levels of physical activity did not significantly increase risk of VF, DHN, or OA, and may protect against OA in men. Differences in the specific type or intensity of activities between women and men may contribute to differences observed in the associations between PAI and spinal degeneration.

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Table 1 [P255]: RR (95% CIs) for the association between physical activity (quartiles) and 6-year incidence of VF, DHN, and OA

	Vertebral Fracture (VF)		Disc Height Narrowing (DHN)		Facet Joint Osteoarthritis (OA)	
PAI	Men	Women	Men	Women	Men	Women
Q1 (low)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
Q2	0.9 (0.3, 2.5)	1.1 (0.3, 3.4)	1.1 (0.7, 1.7)	1.1 (0.8, 1.5)	1.0 (0.7, 1.5)	1.0 (0.7, 1.3)
Q3	1.0 (0.4, 2.7)	1.7 (0.6, 4.5)	1.5 (1.0, 2.1)	1.1 (0.7, 1.4)	0.8 (0.5, 1.2)	1.1 (0.8, 1.5)
Q4	1.1 (0.4, 3.1)	2.0 (0.8, 5.3)	1.4 (0.9, 2.0)	1.1 (0.9, 1.5)	0.7 (0.4, 1.0)	1.0 (0.8, 1.4)
Trend, *p*	0.77	0.08	0.05	0.41	0.03	0.63

P256

Association between Amount of Exercises and Bone Mineral Density Changes During Pregnancy

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Background: The aim was to evaluate the association between bone mineral density (BMD) changes and amount of exercises during pregnancy in low risk women.
Methods: Consecutive patients were recruited from a general obstetric clinic over a period of 9 months. Quantitative USG measurements of BMD were performed at the os calcis bilaterally between 14-20 weeks, and at 36-38 weeks. A questionnaire survey was done on all recruited women at the time of the first BMD measurement. Categorizable exercises included brisk walking, jogging, yoga or Pilates, and formal sports activities. The average amount of exercises performed in the week prior to the survey was recorded.
Results: A total of 390 women were recruited, and the mean BMD loss from early to late pregnancy was 0.0301 g/cm^2, SD 0.043. Within this cohort, 224 (57.4%)(Gp 1) reported exercising less than 1 hour per week, 94 (24%)(Gp 2) between 1-2 hours, 37 (9.5%)(Gp 3) between 2-3 hours, and 35 (8.9%) (Gp 4) reported excising for more than 3 hours. The mean BMD loss was 0.0368g/cm^2 (SD0.036), 0.0408 (SD0.042), 0.0147 (SD0.049) and -0.0247(SD 0.034), respectively for each group, indicating a trend from progressively lower BMD loss to a marginal positive gain with increasing exercise duration ($p<0.005$, ANOVA).
Conclusion: Mild to moderate exercises during early pregnancy of 2 to 3 hours per week at mid trimester and beyond were apparently protective against the physiological fall in BMD during pregnancy compared with those who were sedentary.
Disclosure: The authors declared no competing interests.

P257

Pre-Fracture Medication use as Predictor of 30- And 365 Day Mortality in Hip Fracture Patients

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Background: Hip fractures are associated with increased mortality, which is influenced by several previously described factors, but information on the effect of pre-fracture medication usage is sparse. The purpose of this study was to examine the association between pre-fracture medication and 30- and 365 day mortality following a hip fracture.
Methods: All patients sustaining a hip fracture and admitted to our hospital have since August 2008 been registered in the local hip fracture database. All patients > 60 years admitted in the period September 2008 to January 2012 were identified for this study. The following variables were obtained from the database: habitual medication use, American Society of Anaesthesiologist grading score (ASA-score), body mass index (BMI), age, sex and date of death. Univariate- and multivariate Cox regression analyses were conducted in order to examine the correlation between pre-fracture medication usage and hazard ratio (HR) for death. Adjustments were made for age, sex, ASA-score, BMI, fracture type, operation type and dwelling before admission. A p-value <0.05 was considered statistical significant.
Results: 1404 consecutive patients were included. In the multivariate analyses, a significant increase in 365 day mortality was associated with the use of acetaminophen (HR 1.24) and digoxin (HR 1.38) while 30 day mortality was increased in patients using benzodiazepines (HR 1.60), beta-blockers (1.70) and propulsives (HR 2.19). A significant decrease in 365 days mortality was associated with the use of bisphosphonates (HR 0.56). For statins, a borderline significant (P=0.05) reduction in 365 mortality was found (HR 0.76).
Conclusion: This study shows the correlation between pre-fracture usage of certain medications and mortality following a hip fracture. The information might be used to identify

patients with a higher risk of death and thus result in intensified care in order to reduce excess mortality.
Disclosure: The authors declared no competing interests.

P258

Long-Term Exposure to Microgravity Onboard the Bion-M1 Spaceflight Adversely Altered Skeleton of Mice

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From the beginning of life on earth, gravity has shaped skeleton from cellular to biological function and modelling activity. After a 16-year hiatus, spaceflights onboarding rodents were resumed with the Bion-M1 satellite (April 19-Mai 19 2013) offering a unique opportunity to investigate the effects of gravity on bone tissue. A group of 15-weeks-old C57/Bl16 male mice exposed to spaceflight (Flight, n=5) was compared with a ground synchronous conditions control group for which the environmental conditions of the spaceflight were simulated (Syn, n=6) and a ground standard conditions control group (Ctr, n=6). In femur, animals from Flight and to a less extent from Syn have damaged micro architecture parameters such as BV/TV (%, mean±SEM) (Flight: 1.69±0.4; Syn: 4.80±0.89; Ctr: 11.4±1.72; $p<0.05$) or cortical thickness (µm, mean±SEM) (Flight: 194±4.8; Syn: 205±2.7; Ctr: 215±3; $p<0.05$). At the cortical level, we observe in the Flight group a periosteal resorption, an osteocytic sclerostin expression increased as well as an osteocytic lacunae disorganisation. We also notice an impairment of cortical bone material level properties in the Flight animal vs Ctr; the plastic and elastic properties parameters assessed by nano-indentation are decreased. In T12 vertebrae, BV/TV was also adversely altered (Flight: 22.9±1.9; Syn: 28.5±0.63; Ctr: 30.9±1.46; $p<0.05$), and resorption was highly increased in L3 vertebrae (Oc.S/BS: %, mean±SEM) (Flight: 0.62±0.12; Syn: 0.13±0.03; Ctr: 0.11±0.01; $p<0.01$ between Flight and other groups). In L2 vertebrae, the Flight and Syn animals have a less mature collagen (measured by FTIRM: Fourier Transform InfraRed Microspectroscopy). The degree of mineralisation analysed on micro radiography was unchanged. Intervertebral distances were affected neither by microgravity nor by synchronous conditions. These first data show a major contribution of gravity on skeleton integrity and could contribute to better understand and anticipate the spaceflight-related bone loss in humans.

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P259

Ten minutes of Capoeira Plus Jumping Thrice Weekly Improves Bone and Reduces Fat in Primary School Children: The CAPO Kids Trial

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Introduction: The increasing burden of costly chronic disease warrants novel effective interventions. Many conditions stem from childhood. While exercise benefits both bone and fat, the nature of exercise recommendations for each is different. Development of a simple paediatric exercise program for both bone and fat is therefore indicated. The objective was to determine the effect of a novel, brief, enjoyable, feasible, school-based exercise regimen targeting both bone and fat on body composition of primary school children.
Methods: A nine-month, school-based, controlled exercise intervention trial was conducted (Ethical approval #: PES/25/11/HREC). The intervention (EX) comprised 10 minutes of thrice-weekly capoeira and jumping. Anthropometrics, waist circumference (WC), calcaneal BUA and SI, maximum vertical jump (VJ), cardiovascular endurance (predicted VO_2 max), resting heart rate (HR), blood pressure (BP) and maturity (YAPHV) were examined. A subset of DXA-derived WB, LS and FN BMD, lean and fat mass, and pQCT-derived indices of tibial and radial geometry and density were also collected. Between-group differences were examined using two-way ANOVA, controlling for YAPHV and initial values. Stepwise regressions were conducted to examine the influence of independent variables on outcomes.
Results: 296 children, including 166 EX (10.5±0.5yo; YAPHV -2.1±0.9yo) and 130 control (CON) (10.7±0.6yo; YAPHV -1.9±0.9y) participated. EX improved WC (EX: 1.95±2.82cm; CON: 4.09±4.05cm; $p=0.001$), HR (EX: -4.11±3.35 BPM; CON: 0.22±3.8 BPM; $p=0.001$), VJ (EX: 3.47±4.01; CON: -0.59±5.16; $p=0.001$), predicted VO_2 max (EX: 2.68±3.96; CON:
-0.20±3.38ml/kg/min; $p=0.001$), SI (EX: 6.25±10.04%; CON: 4.09±6.99%; $p=0.05$) and BUA (EX: 3.99±9.06dB/MHz; CON: 1.33±8.3dB/MHz; $p=0.01$), compared with control. Baseline BUA and maturity predicted 32.2% of the variance in BUA change for boys ($p=0.001$). BMI and baseline BUA accounted for 16.4% of the variance in BUA change for girls ($p=0.006$).
Conclusions: School-based capoeira with jumping improved metabolic and musculoskeletal health of primary school children. The exercise program was safe, enjoyable and feasible in the school schedule.
Disclosure: The authors declared no competing interests.

P260

Muscle Size Not Density Predominates Variance in Indices of Bone And Muscle Strength at the Thigh

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Background: While the relationship between muscle size and strength is well understood, the same cannot be said for muscle density. Muscle density from CT is thought to reflect degree of fat infiltration which may impair muscle function. As ageing is associated with both a loss of muscle mass *and* increasing fat infiltration, measures of muscle mass alone may not adequately represent age-related loss in function. Further, the relationship of muscle density to bone strength is not fully understood. Our goal was to determine the relationship between pQCT-derived muscle area (MA) and density (MD) and both muscle and bone strength.
Methods: Ethical approval was obtained (AHS/52/14/HREC). Participants underwent pQCT scans of the dominant thigh (33% from distal) for muscle and bone area and density. Knee extensor strength was tested with an isokinetic dynamometer (Biodex). Linear regressions were used to determine the ability of MA and MD to predict variations in parameters of muscle and bone strength.
Results: 52 participants (33.8±12.0yo) volunteered. MA was highly predictive of muscle strength in all participants, and of bone area and strength in younger participants (24.4±3.1yo). MD was not related to bone density, or to any bone parameter in the older (42.4±10.8yo) participants. (See Table 1.)
Conclusion: As previously observed, thigh muscle size predicts the vast majority of variance in both muscle and bone strength, particularly in young adults. Thigh muscle density has a much weaker relationship with muscle strength, and is only related to indices of bone strength in younger adults.
Disclosure: The authors declared no competing interests. Tom Gerrits was supported by a Travel Award from Radboud University Nijmegen.

P261

Jumping Mechanography versus other Estimates of Muscle Mass and Function in the Prediction of Bone Geometry

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Background: Jumping mechanography has been developed to accurately estimate maximal muscle forces and might therefore be useful when investigating functional muscle-bone relationships. The objective was to determine whether mechanography derived peak jump power or force have greater explanatory capacity concerning bone parameters than other estimates of skeletal muscle derived mechanical loading.
Methods: Data from 181 healthy men (25-45 years) participating in a cross-sectional, population-based sibling study were used. Estimates of mechanical loading included isokinetic peak torque of the quadriceps muscle, DXA-derived leg lean mass (LLM), mechanography derived peak jump power and force and pQCT-derived muscle cross-sectional area (CSMA). Mid-tibial cortical bone parameters were assessed by pQCT. Analyses were adjusted for age, height and weight. Endosteal circumference was additionally adjusted for periosteal circumference (PC) to provide an estimate of endosteal expansion (EC_{PC}).
Results: Jump power and force correlated positively with cortical thickness (ß=0.25,p=0.007 and ß=0.34,p<0.001, respectively) and strength strain index (SSI) (ß=0.30,p=0.003 and ß=0.23,p=0.001), and inversely with EC_{PC} (ß=-0.13,p=0.003 and ß=-0.16,p<0.001). Force but not power was associated with cortical over total bone area ratio (CrtA/TotA) (ß=0.25,p=0.002). CSMA correlated positively with PC (ß=0.21,p=0.001) and SSI (ß=0.26,p<0.001) and inversely with EC_{PC} (ß=-0.09,p=0.016). LLM correlated with all cortical parameters except CrtA/TotA (ß=0.56,p<0.001 for PC; ß=-0.25,p<0.001 for EC_{PC}; ß=0.37,p=0.003 for cortical thickness; ß=0.61,p<0.001 for SSI). Quadriceps torque only correlated with PC (ß=0.21,p=0.023). Based on R^2 statistics, LLM was superior in explaining variation in PC (R^2=0.547) and SSI (R^2=0.562) whereas jump force was superior in explaining variation in EC_{PC} (R^2=0.262), cortical thickness (R^2=0.236) and CrtA/TotA (R^2=0.215), compared with other estimates of mechanical loading.

Table 1 [P260]

		Muscle strength		Bone area		Stress-strain index	
Younger adults	MD	**R^2=0.15**	**P=0.05**	**R^2=0.28**	**P=0.05**	**R^2=0.17**	**P=0.04**
(n=26)	MA	**R^2=0.73**	**P<0.001**	**R^2=0.84**	**P<0.001**	**R^2=0.75**	**P<0.001**
Older adults	MD	**R^2=0.26**	**P=0.01**	R^2=0.01	P=0.98	R^2=0.01	P=0.68
(n=26)	MA	**R^2=0.82**	**P<0.001**	**R^2=0.61**	**P<0.001**	**R^2=0.43**	**P<0.001**
Whole group	MD	**R^2=0.18**	**P=0.002**	R^2=0.06	P=0.08	R^2=0.03	P=0.21
(n=52)	MA	**R^2=0.77**	**P<0.001**	**R^2=0.71**	**P<0.001**	**R^2=0.58**	**P<0.001**

Conclusion: While LLM was the strongest predictor of overall bone size and strength, jump force was the main determinant of cortical bone size, apparently by limiting endosteal expansion. These data indicate that jumping mechanography provides important additional information in the evaluation of muscle-bone relationships.
Disclosure: The authors declared no competing interests.

P262
Association among Sarcopenia and Sarcodynapenia with Osteoporosis in Outpatient Elderly Men and Women
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Background: Sarcopenia and weakness (dynapenia) are associated with disability and falls. Osteoporosis (OP) is associated with fractures and disability. However, there are few studies about the interactions among muscle mass, strength and bone mineral density (BMD). Our aim was to evaluate the association among sarcopenia and sarcodynapenia with osteoporosis among elderly women and men.
Methods: Cross-sectional analysis of Longitudinal study of Sarcopenia and Osteoporosis in Heart Failure Older adults outpatients. Exclusion criteria: dementia, cancer, neurodegenerative diseases, assistant advises. All patients were undergone by DXA analysis: lumbar spine (LS), femoral neck (FN), total femur (TF) and distal radio(DR).Osteoporosis was diagnosed by WHO criteria. Sarcopenia was diagnosed by appendicular muscle mass/Height 2 <5.45 kg/m2 women and <7.27Kg/m2 for men. Sarcodynapenia was considered if sarcopenia was present plus weakness (grip strength <16kgf women and 26 Kgf men).
Results: 122 elderly women(n=70) and men(n=55) were randomised, mean age 80.2 (7,0) and 79,1(6,5) years old, respectively. Osteoporosis was present in 64.1% of women and 33.3% of men. Sarcopenia occurred in 24.3% women and 48.1% of men, while, sarcodynapenia was diagnosed in 16.9% women and 22.8 % of men. Among women sarcopenia and sarcodynapenia were significant more prevalent in patients with osteoporosis of FN (36.8%; p=.20 and 36.8%; p=*.003*, respectively) and TF (50%; p=*.05* and 50%;p=*0.01* respectively). However, among men, sarcopenia occurred in 100% of men with osteoporosis at LS(p=.10), TF(*p=<.001),* FN (*p=.009)* and 87.5% of RD*(p*=.10) *and* Sarcodynapenia occurred at DR(75%, *p=.001*), FN 66.7%,*p=.005*), TF (60%p=.09) and LS(66.7%p=.15). In logistic regression analysis for osteoporosis at any site, adjusted for age, sarcopenia presented OR: 31.94(3.59-283.60;*0.002*) and sarcodynapenia OR:16.01(2.79-91.58;*0.002*) among men however, among women, sarcopenia OR:1.55(0.45-5.30) and sarcodynapenia 2.03(0.048-8.55), did not present significant association.
Conclusions: Our data suggest that there are strong interactions among muscle mass and muscle strength with osteoporosis among elderly men than elderly women, independently, of age. Implications: future studies are necessary to evaluate if the treatment of sarcopenia or sarcodynapenia will interfere in osteoporosis.
Disclosure: The authors declared no competing interests.

P263
Low Bone Mineral Density and Low Vitamin D Levels are Associated With Skeletal Muscle Loss in Mild to Moderate Chronic Kidney Disease (KNHANES 2008-2010)
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Sarcopenia is characterised by the progressive loss of skeletal muscle mass and strength during ageing with various adverse outcomes. In our previous study, we found that stage 3 chronic kidney disease (CKD) is strongly associated with sarcopenia. To date, few studies have investigated sarcopenia in non-dialysis CKD patients. So, we studied the prevalence and the factors associated with sarcopenia in mild to moderate CKD. Using data from the Korea National Health and Nutrition Examination Survey (KNHANES) 2008-2010, we included 1,844 men and 2,264 women aged over 55 years. Sarcopenia was defined as an appendicular skeletal muscle mass(kg)/ height(m^2)< -1SD for the mean of a younger reference group. As a result, the prevalence of sarcopenia was 30.0% in stage 2, 38.4% in stage 3, and 66.7% in CKD stage 4 in man and 5.2% in stage 2, 4.7% in stage 3, and 12.5% in stage 4 in women. In stage 2 CKD, total hip BMD (β=1.172, p=<0.001), vitamin D (β=0.018, p=<0.001), HOMA-IR (β=-0.028, p=0.001) and amount of protein intake (β=0.001, p=0.008) were associated with skeletal muscle mass in man, and total hip BMD (β=1.761, p=<0.001) and vitamin D (β=0.008, p=<0.001) were associated in women. Meantime, in stage 3, only total hip BMD (β=1.940, p=0.028) was associated with skeletal muscle mass in men, and total hip BMD (β=2.492, p=0.001) and vitamin D (β=0.015, p=0.009) were associated with in women. In conclusions, sarcopenia is prevalent in CKD population, and low BMD and low vitamin D levels are associated with low skeletal muscle mass in mild to moderate CKD patients. Early identification and correction of these factors might help to prevent sarcopenia in CKD patients.
Disclosure: The authors declared no competing interests.

P264

The New Concept of Dynapenic Skeletal Muscle Function Deficit in the Assessment of Osteoporotic Patients

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Background: Recent studies demonstrated a significant association between sarcopenia and severe osteoporosis in older women. However, the decline in muscle performance and mobility limitation are due only in part to the age-related reduction of muscle mass. The aim of our study was to evaluate the impact of dynapenic skeletal muscle function deficit (SMFD) on bone fragility in osteoporotic women.
Methods: In this retrospective study, we analysed data derived from the medical record of postmenopausal women aged 55 or older referring to an outpatient rehabilitation service. In our population, we defined dynapenic SMFD according to the Foundation for the National Institutes of Health's (FNIH) criteria, based on reduction of gait speed(<0.8 m/s), handgrip strength (<16 kg), and normal appendicular lean mass adjusted for body mass index (>0.512). We analysed the Vertebral Fracture Assessment (VFA) from DXA spine images to identify vertebral fragility fractures.
Results: Results are showed in table. In our cohort, for a woman with dynapenic SMFD the odds ratio (OR) adjusted for age for vertebral fragility fracture was 1.79 (95%CI = 1.40-3.68; p= 0.044). (See Table 1.)
Conclusions: In our opinion, the concept of dynapenic SMFD could be useful to provide a comprehensive assessment of the risk fracture in osteoporotic patients.
Disclosure: The authors declared no competing interests.

P265

Bivariate Analyses of BMD and Lean Mass in Children Identifies Variants with Novel Pleiotropic Effects Across Six BMD Loci and in the TOM1L2 Locus

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Background: Lean and bone mass are heritable traits with high phenotypic correlation (rho=0.44), likely reflecting the underlying mechanical and biochemical interactions between tissues. Our aim was to estimate the shared SNP-heritability (genetic correlation) of both traits in children and identify genetic determinants displaying pleiotropic effects on lean mass and bone mass accrual.
Methods: Participants make part of two prospective population-based birth cohorts, the Generation R Study (GenR) and the Avon Longitudinal Study of Parents and Children (ALSPAC). GenR children (n=4,071) born in Rotterdam, Netherlands are of multiethnic background with mean age=6.2, SD=0.37 years. ALSPAC children (n=4,820) born in Avon, UK had mean age=9.9, SD=0.32 years. Lean mass and BMD were measured with DXA (GE-Lunar iDXA/ Prodigy) and genome-wide genotyping (Illumina 660K) imputed to HapMap. Shared heritability estimates derived from array data of GenR were obtained using GCTA (with modified admixed-aware relatedness estimates using REAP). GWAS in GenR and ALSPAC were run using PLINK multivariate. Meta-analysis was performed by Fisher's method. All analyses were adjusted for age, sex, height, fat percent (and 20 genomic principal components in GenR). $P<5x10^{-8}$ was considered genome-wide significant (GWS).
Results: SNP-heritability estimates were 0.31 for BMD and 0.40 for lean mass, with a genetic correlation of 0.3. The bivariate GWAS meta-analysis identified GWS associations with concordant effects on lean mass and BMD; mapping to six established BMD loci including: *WNT4, GALNT3, CPED1/*

Table 1 [P264]

	Population (n=70)	Non-dynapenic SMFD (n= 45)	Dynapenic SMFD (n= 25)	p-value
Age	67 ± 7.71	64.73 ± 1.10	71.08 ± 1.34	<0.001
BMI (kg/m^2)	25.21 ± 3.34	25.13 ± 0.52	25.34 ± 0.64	0.80
Falls (n)	13 (18.57%)	8 (17.78%)	5 (20%)	0.82
VFx (n)	36 (51.43%)	17 (37.78%)	19 (76%)	0.002
BMD L1-L4 (g/cm^2)	0.893 ±0.203	0.871 ± 0.031	0.934 ± 0.038	0.23

Note: data are expressed as mean ± SD. BMI= body mass index; VFx= vertebral fragility fractures; BMD= Bone Mineral Density; SMFD= Skeletal Muscle Function Deficit.

WNT16, RANKL, RIN3 and *PPP6R3/LRP5*. Another GWS signal mapping to the *TOM1L2* locus, showed opposite loadings in lean mass (-0.46) and BMD (0.59). ENCODE analyses identified enhancers for *SREBF1* in the same haplotype block.

Conclusion: Several variants at BMD loci exert pleiotropic effects on lean mass. Bivariate analysis is a powerful method for identifying novel pleiotropic effects. *SREBF1* is a regulator of muscle protein synthesis down-regulating *MYOD1*, *MYOG* and *MEF2C* factors. Functional studies are required to unravel underlying pleiotropic mechanisms.

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P266

Botulinum Toxin Injected in Masticatory Muscles of the Rat Induces Bone Loss at the Condyle and Alveolar Regions of the Mandible Associated with a Bone Proliferation at a Muscle Enthesis

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Background: In man, botulinum toxin type A (BTX) is injected in masticatory muscles for several indications such as trismus, bruxism, or masseter hypertrophy. Bone changes in the mandible following BTX injections in adult animal have therefore became a subject of interest. The aim of this study was to analyse condylar and alveolar bone changes following unilateral injections of BTX.

Methods: Unilateral injections of BTX were done in *Mus masseter* and *Mus temporalis* in adult rats. Mature male rats (n=15) were randomised into 2 groups: control (CTRL; n=6) and BTX group (n=9). Rats of the BTX group received a single injection of BTX into right *Mus masseter* and temporal muscles. Rats of the CTRL group were similarly injected with saline solution. Rats were sacrificed 4 weeks after injections. Masticatory muscles examination and microcomputed tomography (microCT) were performed.

Results: BTX rats presented a significant loss of weight ($p<0.05$) from weeks 2 to 4. Atrophy of the right *Mus masseter* and *Mus temporalis* was observed in all BTX rats. MicroCT analysis showed significant bone loss in the right alveolar and condylar areas in BTX rats. Decrease in bone volume reached -20% for right alveolar bone and -35% for right condylar bone. A hypertrophic bone metaplasia at the *Mus Digastricus* enthesis was found on every right hemimandible in the BTX group and none in the CTRL group.

Conclusions: BTX injection in masticatory muscles leads to a significant and major mandible bone loss. These alterations can represent a risk factor for fractures in human. The occurrence of a hypertrophic bone metaplasia at the *Mus Digastricus* enthesis may constitute an aetiological factor for tori.

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P267

Muscle Strength and Physical Performance in Patients with Vitamin D Deficiency

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Background: Many observational studies and some clinical trials suggested a role of vitamin D counteracting bone and muscle loss in aging. Serum levels of 25-hydroxyvitamin D (25OHD) seem to be associated to muscle strength and physical performance. The aim of our study was to compare physical performance and muscle strength in subjects with different serum levels of vitamin D.

Methods: We compared 40 patients with vitamin D deficiency (25 OHD <30 ng/ml) to 37 subjects with normal serum levels of 25 OHD (>30 ng/ml). The muscle strength and muscle performance were, respectively, assessed with the Jamar handheld dynamometer and the Short Physical Performance Battery (SPPB).

Table 1 [P267]

Parameters	Vitamin D deficiency subjects (n=40)	Normal subjects (n=37)	p-value
Age (years)	66.9 ± 7.27	67.54 ± 8.29	NS
Body Mass Index (BMI) (kg/m^2)	25.37 ± 3.59	25.72 ± 3.25	NS
HGS (kg)	12.81 ± 5.10	15.70 ± 6.89	0.038
Gait speed	<1 m/s: 25/40 (62.5%) >1m/s: 15/40 (37.5%)	<1m/s: 20/37 (54.1%) >1 m/s: 17/37 (45.9%)	NS
SPPB score	<8: 17/40 (42.5%) >8: 23/40 (57.5%)	<8: 10/37 (27%) >8: 20/37 (73%)	NS

Results: Results are shown in Table 1.
Conclusion: Subjects with hypovitaminosis resulted to have a significant reduction in muscle strength but not in physical performance.
Disclosure: The authors declared no competing interests.

P268
Vitamin D Status and Muscle Strength among Ethnic Minorities Residing in Northeast Scotland
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Vitamin D may play a role in muscle strength, yet little is known about how this is affected by emigrating from low to high latitudes. This longitudinal study examined the impact of seasonal vitamin D status on muscle strength among people from sunny climate countries (45°N to 45°S to the equator) visiting Scotland (57°N). Ethical approval was obtained for this study. A total of 66 healthy adults (73% Asians (96% Southeast Asians), 15% Africans and 12% Middle Easterns) aged 19-41 years took part; 58% were seen within 3 months of arriving in Scotland. Participants attended visits every 3 months for fifteen months. At each visit, fasted blood samples were collected for analysis of serum total 25-hydroxyvitamin D (25(OH)D) by dual tandem-mass spectrometry. Maximal voluntary contraction (MVC) was measured using a Takei digital grip dynamometer (both arms) and a Biodex dynamometer (right knee extension). Skin type (I-VI) was determined using a CM-2600d spectrophotometer (inner arm). Dietary vitamin D intake and sunlight exposure were assessed by questionnaires. Mean(SD) baseline 25(OH)D was lower in Middle Easterns (16.9(6.8)) nmol/L, p=0.010) compared with Asians (31.1(13.1)) nmol/L and Africans (32.4(10.9)) nmol/L. Mixed model analysis showed that days spent in Scotland was associated with significantly reduced 25OHD (beta=-0.01). Taking vitamin D supplements (beta=10.3), holidays abroad (beta=2.6), higher percentage body surface exposed to sunlight (beta=2.9) and skin type (beta=2.3) were significant positive predictors of 25OHD. There was no association between vitamin D status and mean handgrip strength, although handgrip strength increased over time (first visit=30 kg, fifth visit=32 kg), possibly due to improved technique. For knee strength, the model predicted that for each 10 nmol/L increase in 25OHD, peak torque increased by 2 Nm (p=0.025). Whilst vitamin D status was low among the ethnic minorities residing in northeast Scotland, this condition has a small effect on muscle strength.
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P269
Assessment of Lean Body Mass Deficiency in Ukrainian Women
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Background: The aim of this study was to evaluate the normative data of lean mass in the healthy Ukrainian women.
Methods: 301 women aged 20-87 years (mean age – 57.6±0.9 yrs) were examined. The women were divided into the following age-dependent groups: 20-29 yrs (n=25), 30-39 yrs (n=27), 40-49 yrs (n=22), 50-59 yrs (n=62), 60-69 yrs (n=91), 70-79 yrs (n=59), 80-87 yrs (n=15). The lean and fat masses, bone mineral density (BMD) were measured by the DXA method (Prodigy, GEHC Lunar, Madison, WI, USA). Appendicular skeletal mass (ASM) was measured at all the four limbs with DXA. We've also calculated the appendicular skeletal mass index (ASMI) according to the formula: ASM/height (kg/m2). Low muscle mass values conform to the following definitions: European guidelines (ASMI <5.5 kg/m2) [Cruz-Jentoft A.J. et al., 2010], less than 20% of sex-specific normal population and two SD below the mean of the young adult Ukrainian females (20-39 yrs).
Results: We observed a significant decrease of ASM with age (20-29 yrs – 16.5±0.4 kg, 30-39 yrs – 16.4±0.3 kg, 40-49 yrs – 17.0±0.5 kg, 50-59 yrs – 16.9±0.3 kg; 60-69 yrs – 16.5±0.2; 70-79 yrs – 15.8±0.3; 80-87 yrs – 15.3±0.3; F=2.7; p=0.01). The ASMI values corresponding to a cutoff of low muscle mass by the definitions used were as follows: <5.5 kg/m2 (European guidelines), <5.7 kg/m2 (<20th percentile of sex specific population), <4.8 kg/m2 (two SD below the mean of young Ukrainian females aged 20-39 yrs). The prevalence of low muscle mass in women aged 65 yrs and older based on the above three criteria was 12%, 16% and 1.7%, respectively. ASM was positively correlated with the total fat mass (r=0.20, p=0.0006) and BMD at all sites (BMD of spine (r=0.22, p=0.0002), BMD of femoral neck (r=0.29, p<0.0001)).
Conclusion: Peak muscle mass among the Ukrainian women is achieved in the fifth decade. Appendicular skeletal mass was positively correlated with total fat mass and BMD at all sites.
Disclosure: The authors declared no competing interests.

P270

The Relationship between Eating Behaviour and Bone Mineral Content in Japanese Children

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Background: In previous, eating behaviour has been investigated in studies focusing on a desire for thinness or body shape. However, relation between eating behaviour and body composition has not been studied. In the present study, we investigated the relationship between eating behaviour and whole body bone mineral content (BMC) among Japanese children.

Methods: The subjects were 504 children in 4 elementary schools and 2 junior high schools in Japan. Among these, we obtained cross-sectional data from 401 children (198 boys and 203 girls; age, 10-14 years old) in September and October in 2013. Eating behaviour was assessed by the Japanese version of the Dutch Eating Behaviour Questionnaire for children (DEBQ-C). The DEBQ-C is a 21-item self-rated questionnaire and is divided into three subscales: restrained eating (7 items), emotional eating (7 items), and external eating (7 items). BMC was measured using a single dual-energy X-ray absorptiometry scanner. This study was approved by the internal review board.

Results: In boys, BMC showed positive relationship with a score for external eating of DEBQ-C ($r = 0.222$, $p = 0.002$). On the other hand, in girls, BMC showed positive relationships with a score for restrained eating and emotional eating of DEBQ-C. (restrained eating, $r = 0.154$; $P = 0.028$; emotional eating, $r = 0.146$, $p = 0.037$).

Conclusion: Gender difference was shown in relationship between eating behaviour and BMC.

Disclosure: The authors declared no competing interests. Grant-in-Aid for Scientific Research (B) No. 24370101.

P271

The Association between Life Styles and Bone Mineral Content in Japanese Children

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Background: It is reported that there is an increase in bone mass throughout childhood with a marked acceleration in accumulation at puberty, and physical activity in childhood is valid for bone acquisition. In the present study, we investigated the relationship between various lifestyles and acquired bone mineral content in elementary school children in Japan.

Methods: The subjects were 237 children (boys and girls, 10-12 years old) in 4 elementary schools in Japan. The questionnaire survey and the measurement of bone mineral content were conducted in September and October in 2013. The contents of the questionnaire were bedtime, waking time, hours of sleep, physical activity, and experience of dieting. Whole body bone mineral content (BMC), fat mass, and lean body mass were measured using a single dual-energy X-ray absorptiometry scanner. This study received institutional review board approval, and all subjects provided informed consent.

Results: In boys, BMC was positively associated with experience of dieting ($\rho = 0.329$, $p < 0.001$). Percent of fat mass was positively associated with playing outside ($\rho = -0.350$, $p < 0.001$), physical exercise ($\rho = -0.364$, $p < 0.001$), and positively associated with experience of dieting ($\rho = 0.513$, $p < 0.001$). In girls, BMC was negatively associated with sleeping hour ($\rho = -0.266$, $p = 0.003$), and positively associated with experience of dieting ($\rho = 0.250$, $p = 0.005$). Percent of fat was negatively associated with physical activity ($\rho = -0.260$, $p = 0.004$), and positively associated with experience of dieting ($\rho = 0.275$, $p = 0.002$).

Conclusion: In the present study, related life styles were different between BMC and percent body fat.

Disclosure: The authors declared no competing interests. Grant-in-Aid for Scientific Research (B) No. 24370101.

P272

NO Production by Sodium Nitroprusside in Cruciate Ligament Fibroblasts: the Associated Mechanisms

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Cruciate ligaments (CL) stabilise the joint structure while allowing a very large range of motion. CL injuries in particular are known to lead to development of early osteoarthritis (OA). Oxidative stress and inflammatory reaction may mediate muscle dysfunction by CL injuries. This study is designed to examine the oxidative stress and the modulation in fibroblasts isolated from CL. Sodium nitroprusside (SNP) was used as a NO inducer. The primary cultures of fibroblasts from knee joint CL tissues were treated with SNP at concentrations of 0, 0.1, 0.2, 0.3, 0.4 and 0.5 mM for 24 hours. Measurement of NO was done by Griess reaction. Gene expression and regulation was validated by real-time PCR. Exposure of SNP to fibroblasts showed significant increase of NO production in a dose dependent manner. At the same time, upregulation of iNOS, MMP1 and MMP13, but not MMP2, 3, and 9, was identified. The signal transduction involved the phosphorylation of NF-kappaB transcriptional factor. The selective inhibitor of NF-kappaB, parthenolide, showed inhibition of NO production. Further studies to investigate the antioxidants will be carried out.

Disclosure: The authors declared no competing interests.

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Tibialis Posterior Tenosynovitis Management: Systematic Review

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Background: Tibialis posterior tendon (PTT) is the most powerful invertor of the foot and an important dynamic stabiliser of the mid foot. PTT tendinitis and tenosynovitis have been confused with ankle sprain that led to delay in diagnosis and treatment in the past. There are many treatment modalities available for treating the same ranging from conservative orthotic supports, steroid injections and surgical decompression and debridement. The aim was to systematically review the available literature and provide a summary of the treatment options and its effectiveness in managing posterior tibialis tendinitis and tenosynovitis.

Methods: Literature search was made on the 12th December 2014, on EMBASE, MEDLINE, CINHAL and Cochrane databases using Boolean search terms; Posterior AND Tibialis AND Tendon, Tendinitis, Tendonitis, Tensonynovitis and treatment. Search results were limited to peer reviewed articles from year 2000 to 2014, in English language and on humans. Review articles, case series and case reports were included. Data was extracted with PRISMA guidance and checklist by two independent reviewers and summarised.

Results: Fifty-nine abstracts from search results were reviewed and 9 papers were included based on the inclusion and exclusion criteria for further assessment. There were no RCTs. There was combined pool of 85 patients from the included studies. 49 of them had orthotic support with anti-inflammatory medications. 41 of 49 patients were reported to have 'excellent to good' recovery. Among the surgical group, 15 patients had tendoscopic tenosynovectomy, 19 patients had open surgical debridement and 2 patients had radiofrequency micotenotomy. 13 out of 15 patients had symptoms resolved and full activities resumed with tendoscopic tenosynovectomy while 17 out of 19 patients noticed improvement with open surgical debridement.

Conclusion: Conservative treatment with appropriate orthotic support and steroid/anti-inflammatory medication appears to effective method and in patients who has failed to respond surgical option to be considered earlier to prevent PTT tears and foot deformities.

OSTEOPOROSIS: EVALUATION AND IMAGING

P274 (OP6)

P275 (OP8)

P276 (OP9)

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Incidence and Risk Factors of Subsequent Hip Fractures in Korea: Multicentre Study

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This study analyses the incidence of subsequent hip fractures and its risk factors in the northwestern region of Korea. We analysed hip fracture patients who visited any of the 5 teaching hospitals in the Bucheon and Incheon area from January 2000 to December 2010. Medical records were reviewed and presence of subsequent hip fractures, alcohol history, marital status, live in solitude, dementia, dizziness, American Society of Anesthesiologists (ASA) score, osteoporosis treatment after fracture, body mass index (BMI) and initial bone mineral density (BMD) were analysed. The average follow-up period was 12 months (range: 1-130 months). A total of 2546 patients (female: 1770, male: 776) who had experienced hip fractures were included. Of these, subsequent hip fractures were found in 233 patients (9.2%) (female: 187, male: 46). Mean age at the time of the first fracture was 79.2 years old (range: 50-100 years). The average interval between the first fracture and the subsequent hip fractures was 30.2 months (range: 4 days~154 months). In this large-scale, retrospective, multicenter study, overall incidence of subsequent hip

fractures is 9.2%. Independent risk factors of subsequent fracture are female gender, BMI <22kg/m^2, and being unmarried.
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P278
Diabetes Mellitus Aggravates Cortical Bone Status in Ageing Mice
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The aim of this study was to explore the consequences of diabetes mellitus (DM) on cortical bone status at older age in mice. DM was induced in male CD1 mice (18 months-old) at 3 weeks after streptozotocin injection (45 μg/g BW) (Old-DM). Mice of the same age (Old) or aged 3 months (Young) were included as controls (n=7 each group). Femora were scanned using a GE eXplore Locus μCT scanner (93-μm resolution). Cortical bone analysis was performed at 25% of distal femur height from the growth plate. Blood vessels were identified by lectin staining in paraffin-embedded femoral sections. Mouse tibiae were exposed to 3-point bending evaluation. Tibia microindentation was performed with a BioDent Reference Point Indenter (ActiveLife Science) at the tibia-fibula junction (2N, 10 cycles). Both Old groups showed lower Ct.Th but higher outer and inner perimeter and Bone Marrow Area (BMA), compared with Young ($p<0.05$). Moreover, inner perimeter (mm) and BMA (mm^2) were increased in Old-DM vs Old (mean±SD): 4.61±0.21 vs 4.22±0.42 and 1.40±0.05 vs 1.25±0.04, respectively ($p<0.05$). Old mice displayed reduced work to failure, which further decreased together with reduced strain in Old-DM vs Young ($p<0.05$). Old mice exhibited a trend to lower Total Indentation Distance (TID,μm), 34.08±3.55; Indentation Distance Increase (IDI,μm), 4.71±0.37; and Energy Dissipation (ED,μJ), 3.59±0.45 than Young (35.05±1.56, 4.86±0.43 and 3.92±0.42, respectively); but these parameters in the Old-DM group (TID 34.69±5.52; IDI 5.43±2.44; and ED 4.04±1.67) had larger SD, indicating a more heterogeneous bone behaviour. Loading and unloading slopes was unchanged by age in these mice, but have a trend to decrease or increase, respectively, in Old-DM mice vs Old mice: 0.18±0.03 vs 0.19±0.01 and 0.28±0.05 vs 0.27±0.02 (N/μm). Blood vessel number (#/mm^2) was 17±8 and 10±4 in Old and Old-DM mice ($p<0.01$), respectively, vs Young (29±13). Our results indicate that DM aggravates the compromised cortical bone quality in Old mice, which might increase fracture risk in age-related osteopenia.
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Is TBS Different in Healthy European Caucasian Men and Women?: Creation of Normative Spine TBS Data for Men
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Background: Trabecular Bone Score (TBS, Med-Imaps, France) is an index of bone microarchitectural texture extracted from antero-posterior spine DXA. In this cross-sectional analysis from two facilities in Ukraine and Spain, we have investigated the age-related changes of the lumbar vertebrae microarchitecture assessed by TBS in a cohort of Caucasian men and compare the results to TBS reference data for Caucasian women.
Methods: Subjects in the study were Ukrainian and Spanish men aged 40 and older with a BMD Z-score at spine L1-L4 within ± 2SD. Individuals were excluded if they had fractures, were on any osteoporosis treatment and/or had any illness that would be expected to impact bone metabolism. All data have been obtained from GE-Lunar DXA devices (Prodigy and iDxa, Madison, WI, USA). Cross-calibration between the two centers was performed for TBS. TBS was evaluated at spine L1-L4 but also for all possible vertebrae combinations.
Results: A database of 368 men aged 40 to 90 years was created. TBS and BMD values at L1-L4 were poorly correlated with BMI (r=0.16 and 0.22). TBS was poorly correlated with weight (r=-0.1) and height (0.03) whereas higher correlations were obtained for BMD (r=0.3 and 0.2). TBS values obtained for all lumbar vertebral combinations decreased significantly with age (see figure below, at L1-L4 for men and women). There was a linear decline of 13.5% (~-1.75 T-score) in TBS at L1-L4 between 40 and 90 years of age in men whereas a decline of 16.7% (~-2.58 T-score) was observed in women (Dufour et al.,OI 2012). Conversely to women, there is no modification of TBS decline rate after 65 years in men.
Conclusion: This study established for the first time TBS age related curve in European men at lumbar spine. The decrease seen in lumbar TBS reflects age-related micro-architecture texture changes at spine. Within 40-65 age range, similar TBS decline was observed in both European Caucasian men and women (p=0.8). After 65, TBS decline rate is significantly higher for women than for men ($p<0.01$). This study confirms the need for using gender dedicated reference data.
Disclosure: The authors declared no competing interests.

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Age- and Sex-Specific Bone Structure Patterns Portend Bone Fragility in Radii and Tibiae in Relation to Osteodensitometry: a High-Resolution Peripheral Quantitative Computed Tomography Study in 385 Individuals

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Age- and sex-specific 3D bone structure patterns in human radii and tibiae were investigated with respect to individuals' osteodensitometric classification to unravel associations with site-specific fracture occurrences and underlying loading patterns. A sample of 385 patients (121 men, 264 women, age: 23-91years) was investigated. The patients were classified according to Dual-X-ray-Absorptiometry T-scores in three groups: control (n=60), osteopenia (n=160), and osteoporosis (n=165). Bone architecture and geometry were assessed by high-resolution peripheral quantitative computed tomography (HR-pQCT) of the cortical and trabecular compartments in distal radii and tibiae. We found site-dependent age- and sex-related trends regarding bone architecture and geometry. Females displayed more pronounced age-related changes than males. Specifically, female radii showed both cortical and trabecular structural deterioration with aging, whereas the tibiae demonstrated exclusively cortical deterioration. The mean cortical perimeter revealed a significant age-related increase even after adjusting for body height and weight, which suggests that periosteal expansion can be observed in both the tibia (males: r=0.295, p=0.001; females: r=0.161, p=0.008) and also in the radius (males: r=0.362, p<0.001; females: r=0.136, p=0.024). Statistically significant inter-group differences (age and sex-adjusted) were found in all of the evaluated structural parameters for both the radius and tibia (except for the trabecular thickness and cortical perimeter). Clearly, osteopenia and osteoporosis cases did not reveal higher cortical perimeters in comparison to controls (p>0.05). The tomographic assessment of bone structure further clarifies the architectural basis for increased bone fragility at distal radii and tibiae with advanced age leading to fracture predilection in females. These findings may represent a morphological link to epidemiological data on age-dependent fracture incidences. Our data supports existence of periosteal apposition at the skeletal sites with different loading magnitudes, but not necessarily as a compensatory mechanism to counterbalance bone loss given the lack of differences in periosteal diameter among control, osteopenia and osteoporosis groups.

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Pitfalls in the Interpretation of Bone Turnover Markers in Liver Transplantation

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Background: Osteoporosis and fractures are common in liver disease and fracture incidence increases after orthotopic liver transplantation (OLT). The value of bone turnover markers (BMTs) in the prediction of bone loss and fracture risk pre- and post-OLT is not known.
Methods: The BMTs P1NP, osteocalcin, BALP and CTX were measured initially or in Biobank stored sera at screening and at 3, 6 and 12 months post-OLT in consecutive OLT recipients between 2008 and 2011. A prerequisite was the availability of BMD data and of spinal radiographs at screening and 6 and 12 months post-OLT.
Results: 51 patients (80% male, median age 59yrs) were included. Most common liver pathology was alcoholic (41%) and viral liver disease (26%). At screening, osteoporosis and osteopenia were prevalent in respectively 16 and 33 % at the lumbar spine (LS) and 4 and 44% at the femoral neck (FN), and vertebral fractures were prevalent in 67%. Post-OLT, LS BMD remained stable but FN BMD decreased and 43% of patients developed new fractures. At screening, P1NP and CTX levels were high and osteocalcin levels low, but only CTX levels were predictive for bone loss and fracture risk. An increase in BALP at 6 month post-OLT was predictive for fracture risk a year post-OLT
Conclusion: Despite the many pitfalls in the interpretation of particularly collagen-derived BTMs in liver disease, high CTX levels pre- OLT and an increase in BALP post-OLT were respectively predictive for bone loss and fracture risk during the first post-OLT year.
Disclosure: The authors declared no competing interests.

P282

Impact of Freezer Failure on Bone Marker Stability During Serum Sample Storage

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Bone turnover markers are frequently used as pharmacodynamics biomarkers in clinical studies. Serum samples used to assess bone markers are collected at clinical sites, frozen, and subsequently sent to central laboratories in polystyrene boxes on dry ice. Upon arrival, serum samples are stored in -70°C freezers before being measured in batches. We investigated the stability of the bone markers CTX-I (carboxy-terminal telopeptide of type 1 collagen), PINP (aminoterminal propeptide of type 1 collagen), Osteocalcin, measured on Modular E170, Roche Diagnostics and BSAP (bone-specific alkaline phosphatase), measured on Access II, Beckman Coulter, after a simulation of freezer temperature increase caused by power or equipment failure. Several aliquots of

Table 1 [P282]

Mean values ng/ml	Baseline (-75°C)	8 hrs. (-53°C)	24 hrs. (-45°C)	32 Hrs. (-10°C)	60 hrs. (+4°C for 1 hr.)
CTX-I (p results)	0.462	0.462 (1.0)	0.459 (0.92)	0.461 (0.68)	0.459 (0.20)
P1NP	56.05	56.08 (0.83)	56.01 (1.0)	56.76 (0.40)	55.49 (0.83)
OC	27.59	27.85 (0.92)	27.63 (0.68)	28.14 (0.53)	28.15 (0.17)
BSAP	13.70	13.93 (0.40)	13.51 (0.83)	13.63 (1.0)	13.64 (1.0)

six different serum pools were stored in a full -70°C freezer for 20 days. Then, electricity failure was simulated and the resulting temperature increase was monitored over the following 60 hours. One aliquot of each serum pool was taken out of the freezer after 8, 24, 32 and 60 hours respectively and refrozen at -70°C until analysis. (See Table 1.)
No statistical difference (Wilcoxon test) was observed for the 4 bones markers tested between baseline samples and the follow-up samples at 8, 24, 32 and 60 hours. In conclusion, freezer temperature failure over 60 hours does not affect the validity of the data generated for the 4 bone turnover markers tested.
Disclosure: The authors declared no competing interests.

P283
The Incidence and Risk Factors for the Osteoporotic Fractures in Patients with Chronic Inflammatory Diseases: the GLUCOST Study
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Background: The objectives were to evaluate the frequency and risk factors for osteoporotic fractures in patients with different chronic inflammatory diseases.
Methods: The study was conducted in 34 centers of Russia. 2,342 patients (mean age 53,02±14,03 year, 591 male and 1181 female) completed a special questionnaire (including fractures, duration of the disease, dose and duration of glucocorticoid therapy, major risk factors for osteoporosis, etc.). The patients were divided into two groups according to long-term (3 month or more) oral glucocorticoid (OGC) use: Group 1 - never users (n=1403), Group 2 - current users and ex-users (n=939). Patients in both groups did not differ in age, body mass index (BMI), duration of the underlying disease, family history of hip fractures. The median duration of OGC therapy was 3 years, median daily prednisolone dose - 10 mg.
Results: 9.0% of Group 1 patients and 15.5% of Group 2 patients reported osteoporotic fractures. The patients' age and the duration of chronic inflammatory disease were the significant fracture risks factors in never users group, but in Group 2 the only significant factor was the age. Osteoporotic fracture risk was increased in current and ex-users group compared with never users after adjustment for age, sex, BMI, duration of the underlying disease, family history of hip fractures, tobacco and alcoholic use. Odds ratio for all osteoporotic fractures was 2,2 (95% CI 1,63-3,02, p<0,001), for vertebral fractures - 5,04 (95% CI 2,05-12,37, p<0,001), for forearm fractures - 1,77 (1,10-2,84, p=0,02). The fracture incidence was increased in Group 2 patients of different sex and ages, but the significant risk elevation was demonstrated only in men ≥ 50 years and postmenopausal women.
Conclusion: The age, duration of the disease and chronic oral glucocorticoid use are the main fracture risk factors for patients with chronic inflammatory disease.
Disclosure: The authors declared no competing interests.

P284
Shape and Appearance Models do not Discriminate Hip Fracture Better than Total Femur Integral BMD Alone and are Inferior to Model Combining Direct QCT Measurements of Trochanteric Trabecular BMD and Neck Cortical Thickness
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Background: The purpose of this study was to investigate whether statistical parameters describing the 3D shape of the proximal femur and its local BMD distribution can discriminate subjects with and without acute osteoporotic hip fractures.
Methods: QCT datasets of the femur from 98 postmenopausal women (46 with acute hip fractures) (EFFECT study) were used. Statistical shape models were built by non-rigid registration of segmented femurs to a reference. The shape (appearance) model consisted of principal components, PCs, of the registration displacement vectors (respectively, BMD) for the whole sample set. PCs that discriminated fracture significantly after adjustment for age, height, and weight were included into binary logistic regression to obtain odds ratios and AUC of the ROC curve. A stratified validation (proportional number of ctrl and fx randomly chosen datasets) was performed by means of random partitioning the whole

sample set into k partitions with k=1, 2, and 5. The case k=1 corresponds to the discrimination test on the whole population. The shape/appearance model discrimination was compared with that of total proximal femur integral BMD for the same partitions. Finally, we compared a combined statistical model (both shape and appearance included) with trochanteric trabecular BMD (TrTrabBMD) + cortical thickness of the neck (NeckCortTh).
Results: Both for the appearance and the shape model only one significant parameter was found: third and tenth PC, respectively. The discriminative performance of the appearance model was comparable with that of integral BMD, the shape model was not superior. Performance of the combined shape-appearance model was significantly worse (AUC 0.71 [0.61,0.81]) than TrTrabBMD+NeckCortTh (AUC 0.79 [0.70,0.88]).
Conclusions: The performance of the statistical models was not superior to that of BMD, despite the fact that the model was built from the same dataset (biased optimal conditions). The hypothesised BMD and geometry model with equal number of parameters was much better at discrimination.
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P285

Clinical use of Bone Turnover Markers Beyond the Recommended 3 Month Control after Start of Bisphosphonate Treatment for Osteoporosis

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Background: In osteoporosis, the use of bone turnover markers(BTMs) is recommended 3 months after start of bisphosphonate treatment, but data are scarce, outside the context of clinical trials, on the value of the long-term use of these markers to monitor efficacy of treatment. Our objective was to assess the value of BTMs in the management of osteoporosis beyond 3 months of starting treatment with bisphosphonates.
Methods: Consecutive patients attending the outpatient clinic, aged ≥18years, who started bisphosphonate treatment between 2006-2012 with available baseline and sequential BTMs and BMD data were included. Subgroup analysis was performed when a fracture ≤12months before start of treatment.
Results: 134 patients (98 female), mean age 64.6 years(range 22-89) were included. Sixty-eight (51%) sustained a fracture ≤12 months before start of therapy with median time of 4.5 months. Baseline P1NP and CTX were 57.6±27.6ng/ml and 0.39±0.23ng/ml in the whole group, and 60.4±28.5ng/ml and 0.43±0.25ng/ml in patients with recent fractures. P1NP decreased by 66% (p<0.001) within 12 months, irrespective of a recent fracture. There was a significant correlation between decrease in P1NP at 12 months and increases in lumbar spine(LS) BMD at 12 months(5%,p=0.015) and 24 months(7%,p=0.003), which persisted after adjustment for age, gender, BMI, glucocorticoids, smoking, alcohol use and 25-OH vitamin D levels. In patients with a recent fracture, changes in P1NP at 12 months also significantly correlated with changes in LS BMD at 12 and 24 months (r=-0.309,p=0.046 and r=-0.372,p=0.040).
Conclusion: The significant relationship between changes in BTMs at 12 months and changes in LS BMD at 12 and 24 months after start treatment with bisphosphonates, also observed after a recent fracture, justifies and supports the long-term use of these markers in the management of osteoporosis in the clinic after start of bisphosphonate therapy.
Disclosure: The authors declared no competing interests.

P286

Changes in Multisite Quantitative Ultrasound Speed of Sound over Five Years of Follow-Up: the Canadian Multicentre Osteoporosis Study

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This investigation prospectively described the change in speed of sound (SOS in m/s), as measured by multisite quantitative ultrasound (mQUS), over a five-year period in randomly selected community-based individuals from the Canadian Multicentre Osteoporosis Study (CaMOS). SOS was assessed by Beam-Med Omnisense mQUS at the distal radius (DR) and tibia (TIB). Participants receiving antiresorptive therapy or glucocorticoid therapy were excluded. Participants were grouped by age (<40, 40-49, 50-59, 60-69, 70-79, and 80+ years) to assess age-related differences in the rate of change in SOS. Differences among groups were assessed by analysis of variance. Pearson product-moment correlations were performed between change in SOS and baseline SOS, age, height and mass. In this subset of CaMOS data, there were 321 women (30-87.7 years old) and 201 men (30.2-88.8 years old). The mean (standard deviation) loss of SOS over the five-year follow-up was 94.5 (113.9) and 74.6 (114.8) m/s at the DR and 18.7 (116.3) and 28.1 (100.0) m/s at the TIB in women and men, respectively (between sexes differences: DR p=0.06; TIB p=0.35). For both women and men there were no significant differences in the rate of loss among age groupings, although there was a trend for women to have a greater rate of change at the 50-59 yr group, coincident with the average age of menopause. While there were small, yet significant (p<0.05), correlations between the rate of change in TIB SOS and height (r=-0.10) and mass (r=0.11), the strongest predictor of rate of change in SOS was baseline SOS: DR r=-0.45 and TIB r=-0.47 (both p<0.001). In conclusion, consistent losses in SOS were observed in all age groups over a five-year follow-up. As the strongest predictor of five-year SOS loss was low baseline SOS, individuals with low SOS may have the greatest risk for fracture.
Disclosure: The authors declared no competing interests.

P287

Cortical Measurements of the Tibia from High Resolution Peripheral Quantitative Computed Tomography Images: A Comparison with Micro-Computed Tomography

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HR-pQCT measurements are carried out in clinical research protocols to analyse separately cortical bone and trabecular bone. Micro-computed tomography (micro-CT) is a standard tool for *ex vivo* examination of bone in 3D. The aim of this work was to evaluate cortical measurements derived from HR-pQCT images compared to micro-CT (Skyscan 1172 ® device (voxel size=7.5 µm)) in a distal position with a sufficient amount of cortical bone (4.2 cm from the distal pilon). Sixteen tibia specimens were scanned with HR-pQCT using protocols provided by the manufacturer. The standard measured outcomes included volumetric bone density of the cortical region (Dcomp, mgHA/cm^3), and the cortical thickness (Ct.Th,mm). An auto-contouring process measured cortical porosity (Ct.Po,%), pore volume (Ct.PoV,mm^3), mean pore diameter (Ct.Po.Dm,mm^2),cortical thickness ($Ct.Th_{autoC}$) and cortical Bone Mineral Density (Ct.BMD, mgHA/cm^3). All tibias were harvested in four quadrants at the same position of HR-pQCT measurements (9 mm height) for the micro-CT analyses. Pore volume (PoV), porosity (PoV/TV), pore size (Po.Si), pore spacing (Po.Sp), pore number (Po.N) were measured. micro-CT was compared to HR-pQCT images in site matched areas after averaging the parameters of the 4 quadrants. The correlation coefficients of $Ct.Th_{micro\text{-}CT}$ versus Ct.Th or $Ct.Th_{autoC}$ were high: r= 0.93 p<0.001 and r=0.89, p<0.01, respectively. The other main Pearson correlation (*spearman correlation in italic)* results are in the following Table (*p<0.05, **p< 0.01, ***p<10^{-3}, £ p=0.06)

Table 1 [P287]

HR-pQCT vs. micro-CT Standard Method		PoV	PoV/TV	PoS/PoV	Po.Si	Po.N
	Dcomp	*ns*	-0.92***	0.87***	-0.70*	ns
Auto-contouring process	**Ct.BMD**	*ns*	-0.84**	0.77**	-0.54£	ns
	CtPo	ns	0.69*	ns	ns	ns
	CtPoDm	*0.67**	ns	-0.54£	0.54£	ns
	CtPoV	*0.55£*	ns	ns	ns	ns

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Correlation Between Localised Femoral BMD T-scores and Fracture Site of Hip, and Evaluation of the Sensitivity of FRAX® Probability in Hip Fracture Patients

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Background: We compared T-scores of each femoral neck and trochanteric portion in the femoral neck fracture patients group (NFP) and intertrochanteric fracture patients group (IFP). Our hypothesis is that T-score of neck portion is lower than T-score of trochanteric portion in NFP and vice versa in IFP. We evaluated how FRAX® probability is meaningful and sensitive in hip fracture patients.
Methods: From April 2003 to September 2012, 180 hip fracture patients (98 for NFP group, 82 for IFP group) were included, and the BMD of all patients was evaluated within two weeks after surgery. We evaluated the correlation between localised femoral BMD (T-scores) and fractures site of the hip. We compared the average of T-scores between the neck portion and trochanteric portion in each group. Differences between regional BMD (T-score) of the neck portion and trochanteric portion were calculated in each group. The average of the differences was compared between the two groups. We calculated FRAX® probability in all patients and compared the average of FRAX® probability between NFP and IFP. We evaluated how many patients were included in the high risk group by FRAX® designation, defined as 10-year major osteoporotic fracture probability (MOF) ≥20% or hip fracture probability ≥3%. Our study was approved by IRB.
Results: In NFP, the average T-score in the neck portion was lower (-3.23) than that of the trochanteric portion (-2.55). In IFP, the average T-score of the neck portion was also lower (-2.93) than that of the trochanteric portion (-2.56). These BMD differences between the neck and trochanteric portion in the two groups were statistically significant (p<0.001). In NFP, the average of difference between regional BMD (T-score) of neck portion and trochanteric portion (neck: -3.23, trochanteric: -2.55, difference: 0.68) was greater than the average of difference in IFP (neck: -2.93, trochanteric: -2.56, difference: 0.37) with statistical significance (p=0.001). FRAX probability of MOF in NFP (14.4%) was higher than in IFP (11.1%, P=0.009). FRAX® probability of hip fracture in NFP (8.6%) was higher than IFP. (5.9%, P=0.008) 19.5% of NFP and 10.1% of IFP were classified as high risk group for MOF. 77.3% of NFP and 80.8% of IFP were classified as high risk group for hip fracture.
Conclusion: The average T-score in the neck portion was lower than that of the trochanteric portion in both groups. In NFP, the average difference between T-scores in neck portion and trochanteric portion was higher than IFP. It supports that localised femoral T-scores are relevant to the fracture sites of the hip. High risk group designated by FRAX® probability is meaningful and sensitive tool to evaluate the hip fracture in osteoporotic patients.
Disclosure: The authors declared no competing interests.

P289

Ageing vs Postmenopausal Osteoporosis: Turnover-Independent Bone Composition Maturation Kinetics at Actively Forming Trabecular Surfaces

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Bone strength depends on the amount of bone, typically expressed as bone mineral density (BMD) determined by dual X-ray absorptiometry (DEXA), and on bone quality. Bone quality is a multifactorial entity including bone structural and material properties. Both BMD and bone quality are dependent on bone turnover rates, which change as a function of subject's chronological age. Moreover, the material properties are greatly dependent on tissue age as well. The purpose of the present study was to examine whether bone material properties in health are dependent on subject age, independent of changes in turnover rates, and to contrast them against postmenopausal osteoporosis patients. To achieve this, we analysed by Raman microspectroscopy iliac crest biopsies from: healthy subjects aged 1.5 – 45.7 years, paired biopsies from females before and immediately after menopause aged 46.7 – 53.6 years, and biopsies from placebo-treated postmenopausal osteoporotic patients aged 66 – 76 years. Data were obtained at actively forming trabecular bone surfaces, as evidenced by the presence of fluorescent double labels, and expressed as a function of tissue age. The monitored parameters as a function of subject and tissue age were: the mineral / matrix ratio; the mineral, organic matrix, glycosaminoglycan (GAG), and lipid contents; the mineral maturity / crystallinity; and the relative pyridinoline content. The results indicate that these bone quality parameters in healthy bone are dependent on subject age, independent of bone turnover, suggesting that with advancing age the kinetics of maturation (either accumulation, or post-translational modifications, or both) change. Moreover, they showcase the alterations due to an age-related transition such as menopause as opposed to disease (postmenopausal osteoporosis). The relative pyridinoline content exhibited unique changes in postmenopausal osteoporosis compared to the changes due to healthy ageing. The results of the present study help discriminate between ageing and disease, potentially improving our understanding of osteoporosis.

Disclosure: The authors declared no competing interests.

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Relationship between Serum Levels of Fibroblast Growth Factor 23 (FGF23) and Osteoporotic Nonvertebral Fracture in Postmenopausal Women with Mild Renal Dysfunction

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Background: It has been reported that the presence of high levels of fibroblast growth factor 23 (FGF23) is a risk factor for osteoporotic fracture in elderly men**.** The aim of the present study was to elucidate the association between FGF23 and osteoporotic fracture in postmenopausal women.

Methods: We enrolled 190 postmenopausal women who were undergoing examination for osteoporosis. Serum levels of Ca, P, Cr, PTH, 25-hydroxy vitamin D [25(OH)D], FGF23, PINP and CTX were measured. The BMD of the lumbar spine (L2-4) and femoral neck (FN) was measured using dual-energy X-ray absorptiometry, the presence or absence of morphological vertebral fracture was determined, and the presence or absence of existing non-vertebral fracture was determined through physician interviews.

Results: Mean values of age were 63.4±7.5 years. Mean serum levels of Cr, 25(OH)D, FGF23, PINP and CTX were 0.58±0.10 mg/dL, 16.0±4.2 ng/mL, 33.9±9.1 pg/mL, 53.9±16.6 ng/mL and 0.401±0.149 ng/mL, respectively. Mean BMD value were 0.839±0.149 g/cm^2 (T score -1.6±1.3) at L2-4, and 0.621±0.090 (-1.5±0.8) at FN. FGF23 was not significantly different between subjects with and without vertebral fractures and between subjects with and without nonvertebral fractures. Since FGF23 is linked to renal function, further analysis was conducted by the patients' chronic kidney disease (CKD) stage. In the group of subjects with Stage 2 CKD (eGFR: 60-89 mL/min/1.73 m^2), FGF23 was significantly elevated in subjects with nonvertebral fractures ($p<0.05$), but not in those with vertebral fractures. Logistic regression analysis identified FGF23 as a significant risk factor for nonvertebral fracture, even after adjusted for age, BMI, grip strength, Ca, P, Cr, PTH, 25(OH)D, CTX and BMD [odds ratio: 1.96(95%CI:1.11-3.46), $p<0.05$].

Conclusion: This study showed that FGF23 is a risk factor for nonvertebral fracture in postmenopausal women with mild renal dysfunction.

Disclosure: The authors declared no competing interests.

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Residual Ridge Resorption of the Human Mandible: a Histological and Micro-CT Analysis

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Following tooth loss, residual ridge resorption (RRR) commences. This is a chronic, progressive, irreversible and cumulative process resulting in the decrease of denture bearing area, compromising prosthodontic rehabilitation with complete dentures. Although numerous causative factors have been mentioned, little is known about the changes

in the bone on a cellular level. Therefore, we aimed to investigate the relationship between clinical parameters such as mandibular height (MH) and duration of the edentulous state (DE) with bone histology and micro-CT in mandibular bone of edentulous patients. Twenty-seven patients requiring dental implants for mandibular overdentures, were included, and screened for bone metabolism disorders and DE and MH were registered. During implant surgery bone biopsies were harvested from the mandible. Bone mineral density (BMD) and BV/TV were determined by micro-CT (Scanco medical). Standardised histomorphometry was applied on Goldner and TRAP stained bone tissue sections(NIS elements, Nikon) on cortical and trabecular bone separately. Student's t-test and Spearman correlations were calculated using GraphPad software. DE is negatively correlated with MH (p=0,0135, R=-0,5297 CI -0,7877 to -0,1135). In trabecular bone, BMD was strongly associated with MH (p<0,0001, R=-0,738 CI: -0,890 to -0,438) but not with DE (p=0,142, R=0,332, CI=-0,130 to 0,675). Bone mass indices (BMD, BV/TV) as well as bone formation indices (OS/BS, OV/BV) were not different between men and women while osteoclast number (NOcl/BS) was significantly higher in women compared with men (p= 0.035). Only in women, a significant correlation between DE and osteoclast number was observed (p=0,0153, R= 0,787). In edentulous patients mandibular height is decreasing in time, but only the mandibular height is associated with trabecular BMD. Differences between men and women are observed for osteoclast number, indicating that in edentulous women bone resorption more strongly increases in time compared to edentulous men, possibly resulting in more bone loss.
Disclosure: The authors declared no competing interests.

P292
Improved Accuracy of Vertebral Cortical Thickness Based on CT Data of Different Quality (QCT, HR-QCT And HR-Pqct) by Means of Iterative Convolution Optimisation
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Computed Tomography (CT) permits assessment of densitometric and structural bone characteristics. New therapies may not only improve the cancellous bone, but also strengthen the rather compact cortex, where this is achievable by endosteal or periosteal apposition, increasing mineralisation, or reduction in resorption space. In clinical CT data, limited spatial resolution and hence significant partial volume effects blur thin structures, especially the cortex, which is typically 150-350µm thick (Ritzel 1997). We analysed CT data of 9 excised embedded vertebrae scanned using 3 different CT protocols: (I) HR-pQCT) (Scanco Medical Xtreme CT, voxel size (0,082*0,082*0,082)mm³, 60kV, 190mAs) used as gold standard, (II) HR-QCT (Siemens Sensation64, voxel size (0,156*0,156*0,300)mm³, 120kV, 360mAs) and (III) QCT (Siemens Sensation 64, voxel size (0,234*0,234*1,000)mm³, 120kVp, 100mAs). We developed a method for measuring radial BMD profiles perpendicular to the cortex in a laminar fashion based on an initial cortical segmentation done with Structural*Insight* (in-house development). Using a priori knowledge of vertebral skeletal structure we can fit a BMD profile to the measured data, what results in a deconvolved cortical thickness measure (dcCt.Th). For comparison we also calculated a direct maximum-sphere based cortical thickness (Ct.Th) and, as a simple correction for partial volume effects, weighted cortical thickness (wCT.Th = cortical BMD x CT.Th). Cortical thickness by HR-pQCT was (370±70)µm. Compared with these results the table shows the mean offsets and the root mean square errors (RMSE) of QCT and HR-QCT based estimates. Here dcCt.Th shows very low random residual errors even in QCT data analysis. Our results document that HR-pQCT and QCT both overestimate the cortical thickness by 370% and 320%. This can be reduced for QCT to 40% (dcCt.Th = 434±71)µm and for HR-QCT to 16% (dcCt.Th = 522±93)µm. Variability of the accuracy error was 10% and 5% for QCT and HR-QCT.
Disclosure: The authors declared no competing interests.

P293
Digital X-Ray Radiogrammetry (DXR) Following Mammography Identifies Women at Risk of Osteoporosis by Dual-Energy X-Ray Absorptiometry
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Following fracture, BMD is often assessed by DXA, but cost, availability and time constraints restrict wider screening. DXR evaluation is rapid and can be performed on unmodified digital mammography equipment. This study assesses the correlation of BMD by DXR and DXA, and the potential for DXR screening of women undergoing follow up mammography. Participants were consenting post-menopausal women over age 50. After mammography, an X-ray of their non-dominant hand using an imaging preset was de-identified and transmitted for DXR analysis, and they underwent DXA evaluation at radial, lumbar and proximal femur sites. The mean age of 45 participants was 64±6 years and 77% had been diagnosed with breast cancer. Their BMI was 28.9±5.8, calcium supplements were taken by 42%, vitamin D by 63% and type 2 diabetes or osteoporosis were each reported by 17%. Prior fractures were reported by 33% with 10% falling in the previous year. BMD by DXR and DXA correlated significantly at the 1/3, total and ultradistal radius (r=0.63, r=0.67, r=0.63 respectively; all p<0.001), total hip and femoral neck (r=0.38 for both; P=0.007 and 0.008 respectively) and lumbar spine L1-4 (r=0.45; p=0.001). DXR BMD correlated to forearm BMD more closely than hip or spine BMD by DXA. Neither DXR nor DXA BMD correlated significantly to trabecular bone scores. Predictably, FRAX and Garvan fracture risk results were similar when DXR T-scores were substituted for DXA T-scores (Garvan 5 and 10 year hip fracture risk; r=0.74 for both, p<0.001; major osteoporotic fracture; r=0.76 and 0.75,

p<0.001 for both). The area under the ROC curve for the 1/3 radius BMD by DXR and DXA was 0.84 (95% CI; 0.72-0.96), with DXR T-scores <-1.5 being 85% sensitive and 82% specific for predicting osteopenia/osteoporosis by DXA. Targeted DXR screening identifies women at risk of low BMD by DXA, particularly at forearm sites, and may assist in fracture reduction strategies by identifying patients in need of further evaluation.

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P294

Lateral Lumbar Spine BMD is More Reliable than Conventional Anteroposterior Lumbar Spine BMD in Renal Transplant Recipients at High Risk for Vascular Calcifications

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Background: In renal transplant recipients, fracture risk is high and Bone Mineral Density (BMD) has been shown to be of poor predictive value for this risk. A confounding factor in the interpretation of BMD in these patients is calcification of the large vessels, with conventional anteroposterior (AP) lumbar spine (LS) BMD being potentially falsely increased due to inclusion of calcification in the assessment of BMD. Our objective was to evaluate the impact of vascular calcification on BMD by comparing standard AP LS BMD measurements to lateral LS BMD measurements, in which inadvertent inclusion of calcified large vessels in the area of calculation is precluded.

Methods: Pilot study in consecutive renal transplant recipients aged≥18 years who had lateral LS BMD measurements in addition to conventional AP LS and femoral neck BMD measurements. Vascular calcification was assessed on AP and lateral radiographs.

Results: Sixteen patients (10 female) were included with median age 56.9 years(range19.0-76.7). Mean LS BMD using AP DXA was significantly higher than LS BMD using lateral DXA (0.90g/cm^2±0.15 vs. 0.53g/cm^2±0.13, p<0.001). Mean FN BMD was 0.63g/cm^2±0.11. The difference between AP and lateral LS BMD remained significant, also in the absence of significant vascular calcifications. Using conventional AP LS BMD, one patient had normal BMD, 8 had osteopenia and 7 had osteoporosis. Using lateral LS BMD, only one patient had osteopenia and 15 osteoporosis.

Conclusion: In patients with renal transplantation in whom vascular calcification is common, the significant discrepancy between AP and lateral LS BMD suggests that vascular calcification represents an important confounding factor in the interpretation of LS BMD. Whether eliminating this confounding factor by lateral DXA scans may increase the reliability of BMD measurements and thereby improve fracture risk prediction in this difficult to manage patient group remains to be established by studies including larger numbers of patients and long-term follow-up.

Disclosure: The authors declared no competing interests.

P295

Clinical Aspect of Patients with a Recent Fracture, Presenting at a Fracture Liaison Service

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Methods: All consecutive patients presenting at the Fracture Liaison Service (FLS) with a recent clinical vertebral or non-vertebral fracture were included. Fractures were categorised according to Center (1). DXA was performed and vertebral fractures were assessed. A semi-quantitative scoring was performed according to Genant into grade 0-3. A vertebral fracture (VF) in this study was defined as a VF grade 2 or 3. Laboratory tests were performed [serum calcium, phosphate, 25(OH)D, protein electrophoresis, creatinine, PTH, TSH, and in men < 70 years serum testosterone].

Results: Between May 2012 and October 2013, 945 patients presented with a recent clinical fracture (71.0% women, mean age 65.9 ± 9.9 years). Of 93 patients (9.8%) with a clinical VF, 46.2% had osteoporosis and 40.9% osteopenia. At least one new contributor to SECondary Osteoporosis and metabolic Bone disease (SECOB) was found in 25 patients (27.5%). Of 852 patients presenting with a non-vertebral fracture, 254 (29.8%) had osteoporosis, 438 (51.4%) osteopenia and 160 (18.8%) a normal BMD. Of the patients with osteoporosis, 15.4% had a hip, 19.3% a major, 48.8% a minor and 16.5% a finger/toe fracture. These % were 4.8%, 19.2%, 50.0% and 26.0% in patients with osteopenia and 4.4%, 13.1%, 59.4% and 23.1% in patients with normal BMD. At least 1 VF was found in 153 patients (17.9%), in 26.8%, 15.3% and 11.3% of patients with osteoporosis, osteopenia and normal BMD respectively (p<0.0001). New SECOB was found in 23.5%, 23.8% and 20.0% of patients with osteoporosis, osteopenia and normal BMD.

Conclusion: At presentation at the FLS, nearly 10% of patients had a clinical VF and 27% of them had SECOB. In patients presenting with a non-vertebral fracture, VFs were more frequently found in patients with osteoporosis than in patients with osteopenia or normal BMD. New SECOB on the other hand was present in 23% of all patients, independent of BMD.

Disclosure: The authors declared no competing interests.

P296

Femoral Cortical Index: is the Really Strength of Femur?

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The femoral cortical index (FCI) assesses bone stock using the ratio between the diameter of the femoral shaft and the thickness of the cortical bone calculated 10 cm distal to the centre of the small trochanter in an AP view X-Ray of the femur. It's not clear if low values of FCI may be associated with a condition of altered bone metabolism and bone fragility in fractured patients. The aim of our study is to evaluate a possible association among low values of FCI, risk factors, comorbidities and serum 25 hydroxyvitamin D levels and to establish the importance of FCI as a potential predictor of a new fracture. We conducted a retrospective study on 160 consecutive patients (44 men and 116 women) (range 60 to 103 ya) surgically treated for hip fractures in 2012,after informed consent in our Orthopaedic Department and that never received any medical treatment for osteoporosis. FCI has been calculated by routine clinical radiographs of the pelvis both on fractured femur and on the opposite side. For each patient, we analysed the presence of comorbidities (such as diabetes, hypertension, IRC, rheumatoid arthritis), osteoporosis risk factors and blood levels of vitamin D, usually evaluated in our patients with fragility fractures. Average values of FCI were 0.42 at the fractured femur and 0.48 at the opposite side (range 0.25 to 0.66) with a statistically significant difference. At the fractured side, an average value of 0.45 was found in men, and of 0.40 in women. Patients with severe hypovitaminosis D (serum concentration <12 ng / ml) had a minor FCI compared with those with a moderate deficiency. The presence of comorbidities or osteoporosis risk factors had a different influence on the values of FCI. In our study, we found a correlation among low values of FCI, clinical factors related to bone fragility and severe hypovitaminosis D in elderly patients with hip fractures. Comorbidities and risk factors have a different weight in FCI variations, while the severe hypovitaminosis has a major impact on it. As described in the literature regard the DXA limitations in elderly FCI could be a useful tool in terms of bone fragility evaluation and fracture risk prediction.

Disclosure: The authors declared no competing interests.

P297

Regional Features of Bone Mineral Density in Women At Different Ages

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Background: Osteoporosis is one of the most common non-communicable diseases of modern society. In women, this pathology is found significantly more often than men. Diagnosis of osteopenia and osteoporosis is based on the definition of reduction of bone mineral density in patients. One of the methods allowing to determine bone loss is a dual-energy X-ray absorptiometry. During the DXA results are then compared with the reference base of the bone mineral density (BMD) that was originally put in the densitometric system equipment manufacturer. Often, however, the existing base is different from the reference population BMD patients from other regions. The objective of this study was to perform a comparative evaluation of bone mineral density of the lumbar vertebrae in women Kemerovo region with referential indices database NHANES III.

Methods: The study included 1504 women of the Kemerovo region of different age groups who underwent DXA of the first to fourth lumbar vertebrae. The results obtained BMD women Kemerovo region were compared with the database NHANES III.

Results: On the basis of the study was formed by the reference base indices of BMD of the lumbar vertebrae for the women of the Kemerovo region, taking into account the different age periods. The results showed peak values of bone mineral density of the lumbar vertebrae in women Kemerovo region correspond to the age of 20-29 years, after which, there is a ten-year period of stability indicators. After 40 years of age there is a significant decrease in bone mineral density. Comparative evaluation of the reference values in lumbar spine BMD residents of the Kemerovo region and the values of the database NHANES III revealed no differences in the age groups 16-19 years and 20-29 years. However, after the age of 30 BMD women Kemerovo region were significantly lower ($p<0.05$).

Conclusion: Thus, the use of a reference database developed indicators of BMD for the residents of the Kemerovo region will allow for DXA from a regional perspective that will improve the quality of diagnosis of osteoporosis.

Disclosure: The authors declared no competing interests.

P298

Consistency between AP Spine and Hip T-Score Assessments in a Clinical Population

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Densitometrists routinely find inconsistent T-score assessments when studies are completed on AP Spine and Hip regions. This study investigated T-score assessments of AP Spine and Hip studies in a clinical population to assess expectations and tendencies in regional T-score values. T-score assessments in AP Spine (L2-L4) and Hip (FN, Troc, Wards and Total) were obtained from 1,163 clinical subjects between 21 and 88 years of age using an Norland XR-800. All studies were audited to confirm good methodology. The frequency with which one region identified t-score determined osteoporosis or osteopenia in another region was computed for the various regions. Frequency with which scan sites identify T-score determined Osteoporosis or Osteopenia at other sites. (See Table 1.)

Our studies document inconsistent results in AP Spine and Hip Regional T-score results. The most consistent finding was that an osteoporosis at the Spine, Femur Neck or Trochanter

Table 1 [P298]

	FN	Troch	Wards	Total
AP Osteoporosis	7.8%	53.9%	71.6%	32.0%
AP Osteopenia	42.4%	55.4%	47.5%	41.0%
FN Osteoporosis		74.4%	97.7%	88.4%
FN Osteopenia		46.5%	27.6%	73.0%
Troch Osteoporosis			85.1%	45.1%
Troch Osteopenia			51.0%	64.2%
Wards Osteoporosis				30.8%
Wards Osteopenia				46.8%

was likely to be accompanied with low results in the Wards region. These inconsistent results can be expected given differences in mechanical forces and the trabecular and cortical contents of these sites. In conclusion, AP Spine and Hip assessments can be expected to reveal different results. We therefore suggest attention be paid to the spine and the individual hip regions.
Disclosure: The authors declared no competing interests.

P299

What is the Role of Vertebral Fracture Assessment in the Management of Glucocorticoid Induced Osteoporosis? A Study on 80 Patients

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Background: Glucocorticoid induced osteoporosis is the most common form of secondary osteoporosis. The high risk of fractures and their consequences on back pain and quality of life must induce a previously take of care. A method to detect vertebral fracture (VF) use dual-energy X-ray absorptiometry (DXA), also known as vertebral fracture assessment (VFA). The aim of our study was to evaluate the interest of introducing VFA at the same time of bone densitometry for all patients who begin a glucocorticoid therapy as less as 7,5mg per day for more than 3 months. The patient support was based on the last recommendations of managing glucocorticoid induced osteoporosis in France, GRIO (Group of Research and Information of Osteoporosis) which are quite similar to the European guidelines.
Methods: Transversal study in the university hospital of Limoges, France. Patients with previous VF, discarthrosis or serious scoliosis were excluded. We collected usual risk factors of osteoporosis, comorbidity, and modality of glucocorticoid therapy with a questionnaire and obtain the BMD measurements and T-score (neck of hip and lumbar spine) with a bone densitometer (Lunar iDXA). All VFA were analysed by two experimented readers. We used both a qualitative and semi-quantitative method (Genant) for the diagnosis of VF. In a second time, a radiography was made on patients with VF on VFA by the second reader to confirm VF and exclude a malignant aetiology.
Results: Eighty patients were included in the study: 48 women and 32 men. The mean age was 68.5 years. 11 patients had a least one VF on VFA according to the second reader. Spine radiography confirmed VF on 7 patients. Among these patients, one could obtain a treatment thanks to the presence of VF. He was a 67 years old man with T-score > -1and FRAX under the age range without any history of fragility fracture. 2 patients had an indication to teriparatide with 2 VF on VFA. Total: 10 unknown VF were diagnosed, with prevalence of VF at 8.75%. In VF group, there was significantly more lumbar colon osteoporosis and most women. PAKAB was at 0.44 (95IC 0.22-0.68).
Conclusion: Only one patient in our study had an indication to anti osteoporosis's treatment while he hadn't before VFA. Two patients had a change of their treatment with teriparatide. VFA allowed a change in management of glucocorticoid-induced osteoporosis in 3 patients. Prevalence of VF was lower than literature knowledge partly because VF was an exclusion criteria in our study. Difference between two readers was on men's VFA and mild VF that is also known in the literature. VFA may overstate the prevalence of mild VF. VFA at baseline of management of glucocorticoid-induced osteoporosis change our support in less of 5%. But VF on VFA is very important for patient's monitoring as absence of VF.
Disclosure: The authors declared no competing interests.

P300

Time-Lapse *In Vivo* Image Analysis to Determine Local Disease and Treatment Effects on Bone Remodelling in Patients

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In the clinic, bone disease and treatment are usually evaluated using bone remodelling markers and bone density measurements. In some cases where local microstructural changes occur, however, these measures might not be able to capture the actual effects. In a previous study, vibration therapy in osteopenic girls with adolescent idiopathic scoliosis (AIS) showed no effect on distal tibia bone density and microstructure although this would have been expected at this load-bearing site. Recently, an image analysis approach was developed to determine local sites of bone remodelling in patients based on high-resolution peripheral quantitative computed tomography. For the purpose of this study, we applied the newly developed image analysis method to

the follow-up scans of five osteopenic AIS patients receiving vibration therapy and three controls receiving observation only. In agreement with the clinical outcome, we did not find any difference in global bone remodelling measures between the groups. However, the local visualisation of bone remodelling sites indicated cortical drift, where bone was added on the outside and removed on the inside of the cortex in the treatment group whereas this was less pronounced or even the other way around in the control group. Such a drift might increase mechanical integrity without affecting global bone density or microstructural parameters and thus is only detectable with a local measurement. However, results should be interpreted with caution as they are limited by a small number of subjects and confounded by growth in the still growing subjects. Most of the subjects showed considerable bone turnover, which in one severe case complicated accurate registration of the follow-up images. Nevertheless, we conclude the image analysis approach for local bone remodelling is promising for evaluating disease and treatment in more detail and using a more local approach.
Disclosure: Bert van Rietbergen is a consultant for Scanco Medical AG. The other authors declare that they have no competing interests.

P301
Clinical Efficacy of the Korean FRAX® Model According to the BMD Value in Osteoporotic Fracture Risk Prediction
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Background: As a criterion for selecting an osteoporosis treatment target, this study investigates the effectiveness of clinically using the Fracture Risk Assessment Tool (FRAX®) developed by the World Health Organization (WHO) and evaluates changes in osteoporotic fracture risk prediction according to the bone mineral density (BMD) values of lumbar spine and hip.
Methods: Osteoporotic fracture occurred in 531 of 4556 patients who underwent hip and lumbar spine BMD measurement at our hospital from April 2003 until March 2013. Of the 531 patients, we excluded those who had no BMD measurements within 2 weeks of fracture, those who had no risk factor data needed to calculate the FRAX® value, and those who were already undergoing osteoporosis treatment. Finally, we analysed the FRAX® value in 445 patients by inputting the BMD values of lumbar spine and hip. In accordance with the standards of the National Osteoporosis Foundation (NOF), FRAX® values greater than 20% for major osteoporotic fracture or 3% for hip fracture were considered high risk.
Results: Among the 445 patients, the high risk group consisted of 330 patients (74%) when lumbar spine BMD was used to calculate the FRAX® value, 281 patients (63%) when hip BMD was used, and 258 patients (58%) when no BMD values were used. For the 84 patients with osteopenia, using the lumbar spine BMD in the model did not lead to a significant difference in the average FRAX® value compared with when hip BMD was used. However, the average FRAX® value was significantly higher when no BMD values were used in the model ($p<0.001$). For the osteopenia patients, the high risk group consisted of 39 patients (46%) when BMD was not used, 19 patients (23%) when hip BMD was used, and 14 patients (17%) when lumbar spine BMD was used. The highest osteoporotic fracture probabilities occurred when BMD was not used.
Conclusion: Clinicians using the FRAX® model to determine osteoporosis treatment for patients with osteopenia may be able to improve clinical efficacy by excluding BMD values from the model.
Disclosure: The authors declared no competing interests.

P302
Prevalence of FRAX Clinical Risk Factors Dietary Calcium Intake Habits and Osteoporosis Screening in Rural Area Men
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Background: Worldwide, approximately one in five men aged 50 years or over will suffer an osteoporotic fracture. In rural Greek areas, access to the nearest DEXA facility combined with a very low male referral rate, make their osteoporosis burden assessment more difficult. Our aim was to estimate, for the first time in our area in men, the prevalence of FRAX clinical risk factors (FRF), calcium intake lifestyle habits and perform osteoporosis screening in 101 men, aged 50-84 years.
Methods: Our study was carried out in our municipality and included FRF evaluation by our trainees, BMD measurement with heel QUS and dairy calcium intake using a food frequency questionnaire.
Results: Mean age: 68,02 years, BMI: 27,52 kg/m². We found that 1 and 2 out of 51 (aged 50-70) and 50 (aged over 70) men had T>-2,5. Accordingly, 34 (68%) aged 50-70, and 18 (36%) aged over 70, had T<-1. Also 3.9% and 68% had hip fracture probability over 3% without BMD for the age groups 50-70 years and over 70 years, respectively. Their average calcium intake from dairy products was 480 and 438 mg for

Table 1 [P302]

Age Group	History Of Hip Fracture	Parent fractured hip	Smoking	Alcohol	Secondary Osteoporosis	NO FRF
50-70 N= 51	5	11	15	15	2	16
>=70 N= 50	3	11	3	2	11	26

the age groups 50-70 and over 70 years, respectively. See Table 1 for our results.
Conclusions: In our study we found that the most common FRF are smoking and alcohol for the age group 50-70. Also secondary osteoporosis and parent fracture hip for the aged over 70. Their average calcium intake from dairy products is far from adequate but it is compensated partly from their Mediterranean diet habits. Further actions are necessary to tackle with this vastly underestimated and neglected health issue.
Disclosure: The authors declared no competing interests.

P303
Age Related Changes on Vertebral Cross Sectional Size
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Vertebral cross sectional size is reported to stay relatively unchanged throughout most of adulthood. However, several studies have indicated clear, age-related trends in vertebral size as in elderly age periosteal apposition results in increased size and osteoporosis reduced bone mineral density (BMD) of vertebral corpus. Observed changes in vertebral size are reported to be sex-specific as elderly males have more pronounced increase of vertebral cross sectional area (CSA). As age-related reduction of BMD occurs within both sexes it is suggested that elderly women have increased risk for vertebral fractures. In this study, we wanted to explore whether the proposed, age related trend in vertebral size, have as clear sex specific differences as suggested. To conduct this study we utilised data from two lumbar spine magnetic resonance imaging (MRI) samples (n=497) with age range of 21 to 80 years. Vertebral CSA was determined measuring three width and length dimensions from the corpus of the fourth lumbar vertebra (L4). Our results indicated only moderate association between age and vertebral CSA. We couldn't detect any sex specific trend in this association. According to our observations there are no clear sex-specific compensation mechanisms for age related bone loss in vertebral size.
Disclosure: The authors declared no competing interests.

OSTEOPOROSIS: PATHOPHYSIOLOGY AND EPIDEMIOLOGY

P304 (OP10)

P305 (OP11)

P306
Analysis of Osteoimmune Interactions in Transgenic Mice Overexpressing Human RANKL
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Receptor activator of nuclear factor-κB ligand (RANKL) is a central regulator of bone remodelling by mediating osteoclast-induced bone resorption. We have recently generated transgenic mice (TghuRANKL) carrying the human RANKL (huRANKL) genomic region and achieved a physiologically relevant pattern of RANKL overexpression. TghuRANKL mice of both sexes developed early-onset bone loss and the levels of huRANKL expression were correlated with bone resorption and disease severity. Low copy Tg5516 mice expressing huRANKL at low levels displayed a mild osteoporotic phenotype as shown by trabecular bone loss and reduced biomechanical properties. Notably, overexpression of huRANKL, in the high copy Tg5519 line, resulted in severe early-onset osteoporosis featured by lack of trabecular bone, destruction of the growth plate, increased osteoclastogenesis, increased cortical bone remodelling and severe cortical bone porosity accompanied by decreased bone strength. Anti-RANKL therapies fully corrected the osteoporotic phenotypes of TghuRANKL mice, promoting their significance for the preclinical evaluation of novel drugs targeting RANKL. Apart from the skeletal system, RANKL is also expressed in lymphoid tissues. Analysis of the Tg5519 immune profile demonstrated severe leukopenia in the bone marrow (BM) followed by a parallel increase in BM adiposity. Moreover, an increase of the percentage of B lymphocytes was identified in the spleen of Tg5519 mice, similarly to other osteoporotic mouse models. The involvement of lymphocytes in the osteoporotic phenotype is currently under investigation using reciprocal BM transplantation experiments. In addition, we investigated the role of RANKL in the progression of inflammatory arthritis, by crossing Tg5519 mice with the Tg197 TNF-driven mouse model of arthritis. Our results showed that the simultaneous overexpression of RANKL and TNF resulted in an aggressive inflammatory phenotype characterized by accelerated pannus formation and bone erosion. These results reveal an interaction between skeletal and immune systems during osteoporosis and imply a modulatory role of RANKL in inflammatory arthritis.
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P307

Trabecular Bone Score (TBS) Predicts Major Osteoporotic Fractures (MOF) and Hip Fracture (HF) in Older Men Independent of Prevalent Radiographic Vertebral Fracture and FRAX: Findings from the Osteoporotic Fractures in Men (MrOS) study

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Background: It is unknown if TBS predicts incident fractures in men independent of prevalent radiographic vertebral fracture (PVFx)*. Our purpose was to estimate the association of TBS with incident MOF (hip, clinical vertebral, wrist, or proximal humerus) and HF in men after adjustment for; a) PVFx and, respectively, 10 year MOF or HF risk by FRAX with BMD; and b) PVFx and individual clinical risk factors.

Methods: TBS was estimated on AP spine DXA scans obtained at the baseline visit for 5,946 men age 65 years enrolled in MrOS. Incident HF and MOF were ascertained by self-report every 4 months and confirmed by review of radiograph reports. The multivariable adjusted hazard ratio (HR) of incident HF and MOF over 10 years follow-up per standard deviation decrease of TBS was estimated with Cox regression.

Results: TBS was associated with a 1.2-fold increased risk of MOF adjusted for PVFx and either 10 year FRAX MOF risk or clinical risk factors. TBS was also associated with similar increase in risk of HF adjusted for PVFx and 10 year FRAX HF risk, but not with HF adjusted for PVFx and individual clinical risk factors. (See Table1.)

Conclusion: TBS predicts incident fractures in older men independent of PFVx and FRAX 10 year risks, and these data support development of algorithms to adjust estimated FRAX fracture risks for TBS. TBS does not predict incident hip fracture adjusted for PVFx and individual clinical risk factors.

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Table 1 [P307]

Covariates	HR per SD TBS decrease (95% C.I.)	
	MOF	HF
PVFx* & FRAX Risk	1.24 (1.14, 1.35)	1.24 (1.08, 1.42)
PVFx & Individual Clinical Risk Factors^	1.27 (1.12, 1.44)	1.09 (0.89, 1.33)

*One or more SQ grade 2 or 3 vertebral fracture(s)
^Age, femoral neck BMD, prior fracture, parental history of hip fracture, body mass index, rheumatoid arthritis, glucocorticoid therapy, alcohol use, smoking status

P308

Self-Estimated Health Predicts Hip Fracture Risk Independent of All Risk Factors in FRAX

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We may still only assess fracture risk with moderate accuracy. This is in spite of several new visualisation techniques, biochemical markers and risk-factor based calculation tools. There are thus probably additional factors that affect fracture risk, to be found. Bad luck is probably not the only explanation. FRAX is the fracture prediction tool that is most widespread worldwide. FRAX includes BMD of the femoral neck along with eleven other risk factors for fracture. The aims of this study was to see if self-estimated health measured with a visual analog scale (VAS) was associated with hip-fracture risk. In 1999, 351 Swedish women aged 69-79 years at inclusion, were included in a population-based prospective cohort study. They estimated their global health by putting an “x” on a 100 millimeter line drawn between “worst imaginable health” and “best imaginable health”. At the same visit they were also assessed with FRAX including bone mineral density (BMD). Ten years later, Swedish medical records were used for follow-up regarding fractures and mortality. The main outcome was a hip fracture. No participant was lost to follow up. During a median follow up time of 9.8 years, 40 participants (11%) had a hip fracture. The age adjusted hazard ratio (HR) of a hip fracture was 3.70 for the lowest quartile of self-estimated health compared to the highest quartile of self-estimated health. This relation was unaffected by adjustment for BMD as well as for any other single risk factor of the risk factors included in FRAX. Not even adjustment for all risk-factors in FRAX together, altered this relation between self-estimated health and hip fracture risk. However, adjustment for maximum one-leg standing time, made the relation become non-significant! Self-estimated health thus seems to say something about hip-fracture risk not covered by any of the clinically most commonly considered risk-factors.

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P309
Hip Fractures in Patients with Dialysis: the Impact of Dementia

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Background: Hip fractures (HF) is associated with significant morbidity and is further increased in patients with chronic renal failure (CRF). Higher morbidity in dialysis patients is related to several risk factors, in particular dementia. We here aimed to describe the impact of dementia in FH in dialysis patients and assess if CRF is an additional risk factor of HF in patients with dementia.
Methods: Data are obtained from the National Database of Hospitalizations over the period 2011-2013. Three populations of subjects aged 60 and over were extracted and analysed: population with hip fracture, population in dialysis and population of demented patients (whatever the reason for hospitalisation in connection or not with dementia). These populations were crossed to estimate fracture risk based on the presence of dementia or dialysis, adjusted for age and sex. The fracture risk was calculated using a multiple logistic regression model.
Results: Over the period 2011-2013, 213 180 patients had a HF (70% women), 660 434 patients were diagnosed for dementia (64% women) and 47 430 patients were on dialysis (39% women). There was a strong effect of age and gender in the incidence of fractures and dementia. In dialysis patients, the risk of HF was higher in patients with dementia than without dementia: OR 1.99 [95% CI: 1.66-2.38], this being the same for men OR 2.37 [1.81-3.05] and women OR 2.56 [1-97-3.29] regardless of the age. In patients with dementia, the fracture risk is independent of dialysis OR: 1.25 [0.99-1.59] in each sex and age categories. However, the risk of dementia is not increased by dialysis in this population with HF OR 1.52 [0.77-3] after adjustment for age and sex.
Conclusion: Dementia significantly increases the risk of hip fracture in dialysis patients, but the risk is the same in dialysis patients with dementia than non dialysis patients with dementia. These results highlight dementia as a major risk factor for FH in dialysis.
Disclosure: The authors declared no competing interests.

P310
Effect of Calcium Intake on the Interrelationship between Bone Turnover and Energetic Metabolism in Genetically Predisposed Obese Rats

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Background: According to literature, bone, fat mass and pancreas are interrelated through osteocalcin (BGP), bone resorption, leptin and insulin levels. Ca intake might affect these interrelationships by inducing changes in bone resorption. Obesity could also affect bone and pancreas interrelationship through leptin effect on the osteoblastic insulin receptor or in sequestering vitamin D in fat pad. The present report evaluated changes in these interrelationship by feeding a low, normal or high Ca diet (LCa NCa and HCa, respectively), in growing genetically predisposed obese IIMb/β (O) rats.
Methods: Female O adult rats were mated and fed during pregnancy and lactation diets prepared according to AIN´93 recommendations that only varied in Ca content (0.2%, 0.5% and 0.9%). At weanling, male pups continued feeding the same diet *ad libitum* until 50 days of age. Food consumption and body weight (BW) were recorded 3 times/week. Serum Ca, phosphorus (P), BGP, CTX, 25OHD, glucose and insulin, and total body fat and adipose perigonadal and retroperitoneal percentage (PG+RP %) pads were determined.
Results: Results (mean± SE) are shown in Table 1.
Conclusion: The results suggested that both, Ca intake and dietary Ca/P ratio influence bone, fat mass and pancreas interrelationship in genetically predisposed obese IIMb/β rats. UBACyT 20020100100320/2011.
Disclosure: The authors declared no competing interests.

Table 1 [P310]

	Olca (N=10)	ONCa (n=11)	OHCa (n=10)
sCa (mg/dl)	10.4±0.2	10.3±0.3	10.6±0.3
sP (mg/dl)	11.9±0.7	10.5±0.6*	10.9±0.4*
BGP (ug/ml)	514±60	375±46*	739±191*,**
SCTX (mg/ml)	70±4	83±7*	69±13**
Insulin (mg/dl)	6.9±1.2	4.1±0.8*	1.9±1.3*,**
Glucose (mg/dl)	252±49	152±69*	112±62*,**
25OHD (ng/mL)	24.8±2.4	19.0±2.7*	20.5±1.1*
Body Fat (g/100 BW)	15.9±1.5	13.1±2.2*	12.6±2.2*,**
PG+RP%/BW	5.34±0.24	4.36±0.48*	3.77±0.46*,**

(* and **): p<0.05 vs. OLCa and ONCa, respectively (ANOVA-Bonferroni). No significant differences were observed in sCa among the three O groups. OLCa presented the significantly highest P, insulin and glucose levels. CTX levels were lower in OLCa and OHCa than in ONCa. BGP levels increased as follow: OHCa >OLCa> ONCa. Fat and PG+RP% were inversely related to dietary Ca content.

P311

Current Characteristics of Bone Metabolism Disorders in Liver Transplant Candidates

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Background: Liver transplant (LT) candidates have metabolic bone disorders that may influence the development of skeletal fractures after LT. Our aims were to analyse bone-disorder characteristics in patients included in a screening pretransplant program for preventing bone disease after LT.
Methods: We analysed the clinical and laboratory data of LT candidates included in the screening programme, from 2010 to 2014. Lumbar and femoral BMD (DXA) and spinal X-rays were also evaluated.
Results: Three hundred and forty-five LT candidates (M/F:253/92, age:55.4±9.8 years) were included. Eleven patients had cholestatic liver diseases, 87 had alcoholic cirrhosis, 168 had cirrhosis of viral aetiology, and the remaining 79 had mixed liver diseases. 142 patients had a hepatocellular carcinoma (HCC). Eleven HIV-positive LT candidates were also evaluated. 29% of patients had densitometric osteoporosis and 48% osteopenia. Moreover, 24% of them had associated skeletal fractures. 25-OHD levels were <20 ng/ml in 83% of patients. When evaluating by gender, female patients showed higher prevalence of fractures ($p<0.05$), lower lumbar and femoral BMD (gr/cm^2) ($p<0.05$) and higher 25-OHD values ($p<0.05$) than male patients. Nevertheless, no differences between sexes were found in the prevalence of osteoporosis or osteopenia. When evaluating by liver disease, patients with HCC showed less severe hepatic disease ($p<0.001$), lower prevalence of skeletal fractures ($p<0.05$) and higher BMD ($p<0.05$) and 25-OHD values ($p<0.001$) than non-HCC patients, whereas HIV-positive patients shower a higher prevalence of osteoporosis ($p<0.001$) and lower femoral BMD values ($p<0.05$) than non-HIV patients.
Conclusions: Liver transplant candidates show an elevated prevalence of fractures, low bone mass and vitamin D deficit. Low bone mass and fractures are more frequent in female patients; and vitamin D deficiency is more common in male patients. Bone metabolism disorders are less severe in LT candidates with hepatocellular carcinoma, while HIV-positive patients have high risk of osteoporosis.
Disclosure: The authors declared no competing interests.

P312

Sclerostin and Dickkopf1 (DKK1) Expression in Bone in Relation to Bone Metabolism

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Background: Osteocytes produce sclerostin and DKK1. Sclerostin and DKK1 are well known antagonists of the canonical wnt signalling pathway and thereby inhibitors of osteoblast function. At tissue level, the role of DKK1 and sclerostin expression on bone structure and turnover is not well established. The aim of this study is to identify the relationship between DKK1 and sclerostin expression in bone tissue and markers of bone formation and bone structure.
Methods: Men (n=18) and women (n=20) with quiescent Crohns disease and DXA-assessed osteopenia participated in this double blind clinical trial (NTR 163). Transiliac bone biopsies were obtained. Sclerostin and DKK1 expression was detected using immunohistochemistry and quantified by detection of positively stained osteocytes as a percentage of total number of osteocytes/mm2. Standardised histomorphometry was performed to analyse bone formation and structure. A Pearson correlation coefficient was calculated to test the association between sclerostin and DKK1 expression and histomorphometric indices for bone mass and turnover.
Results: No significant correlation between sclerostin and DKK1 expression in trabecular bone ($r = -0,17$ $P = 0,29$) was found. In patients with a higher bone volume sclerostin expression in trabecular bone osteocytes was higher ($r = 0,53$ $P = 0,0007$) while DKK1 expression decreased ($r = -0,36$ $P = 0,03$). DKK1 expression in trabecular bone osteocytes was negatively associated with bone formation rate: $r = -0,36$ $P = 0,03$.
Conclusions: Although sclerostin and DKK1 are both inhibitors of osteoblast differentiation, their relationship with bone volume is reversed. Possibly high levels of sclerostin inhibit DKK1 expression either directly or indirectly via lower bone formation, however an association between sclerostin and DKK1 expression was not observed. Only a higher DKK1 expression was associated with a lower bone formation. In conclusion, our study suggests that sclerostin expression reflects mainly bone mass while DKK1 expression represents mainly bone formation.
Disclosure: The authors declared no competing interests.

P313

Dietary Patterns in an Elderly Population with High Dairy Intake and their Relation with Bone Mineral Density: the Rotterdam Study

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Background: It is unclear whether overall dietary patterns influence bone mineral density (BMD) in populations with a relatively high dairy intake. The objective was to identify dietary patterns that are associated with BMD in Dutch middle-aged and elderly subjects.
Methods: Participants were subjects 50 years and over (n= 5435) from The Rotterdam Study, a population-based prospective cohort study. Baseline intake of 28 pre-defined food groups was determined by a validated food frequency questionnaire. Dietary patterns were identified using Principal Component Analysis on these food groups. BMD of the femoral neck was measured by Dual Energy X-ray Absorption at baseline and at three subsequent visits between 1993 and 2004. Linear mixed modelling was used to analyse adherence to each pattern with repeatedly measured BMD (both in Z-scores). Results were stratified by status of body weight change.

Results: After adjustment for potential confounders related to lifestyle, socioeconomic status and prevalent chronic diseases, two dietary patterns were associated with high BMD. A "Traditional" pattern, characterised by high intake of potatoes, meat and fat (β= 0.08; 95% CI: 0.05, 0.11) and a "Mediterranean-like" pattern, characterised by high intake of fruits, vegetables, poultry and fish (β= 0.06; 95% CI: 0.04, 0.09). The "Processed" pattern, characterised by high intake of processed meat and alcohol was associated with low BMD (β= -0.03; 95% CI: -0.06, -0.01). The associations of the "Mediterranean- like" and "Processed" pattern were independent of body weight. Furthermore, no effect modification by body weight change was observed.
Conclusion: In a population with relatively high dairy intake, a "Mediterranean-like" and "Traditional" dietary pattern may have additional benefits for BMD whereas adherence to "Processed" dietary pattern may pose a risk for low BMD.
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P314

The Association between Metabolic Syndrome, Bone Mineral Density, Hip Bone Geometry and Fracture Risk: the Rotterdam Study

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The association between metabolic syndrome (MS) and bone health remains unclear. We aimed to study the association between MS and hip bone geometry (HBG), femoral neck bone mineral density (FN-BMD), and the risk of osteoporosis and incident fractures. Data of 2040 women and 1510 men participants in the third visit (1997-1999) of the Rotterdam Study (RSI-3), a prospective population based cohort, were available (mean follow-up 6.7 years). MS was defined according to the recent harmonised definition. HBG parameters were measured at the third round visit whereas FN-BMD was assessed at the third round and 5 years later. Incident fractures were identified from medical registry data. After correcting for age, body mass index (BMI), lifestyle factors and medication use, individuals with MS had lower bone width (= -0.054, *P*=0.003), lower cortical buckling ratio (β= -0.81, *P*=0.003) and lower odds of having osteoporosis (odds ratio=0.56, *P*=0.007) in women but not in men. Similarly, MS was associated with higher FN-BMD only in women (β= 0.028, *P*=0.001). In the analyses of MS components, the glucose component (unrelated to diabetes status) was positively associated with FN-BMD in both genders (β= 0.016, *P*=0.01 for women and β= 0.022, *P*=0.004 for men). In men, waist circumference was inversely associated with FN-BMD (β= -0.03, *P*=0.004). No association was observed with fracture risk in either sex. In conclusion, women with MS have higher FN-BMD independent of BMI. The glucose component of MS was associated with high FN-BMD in both genders, highlighting the need to preserve glycaemic control to prevent skeletal complications.
Disclosure: The authors declared no competing interests.

P315

The Predictive Value of Haemoglobin at Admission on 30-Days Mortality in Hip Fracture Patients

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Background: Previous smaller sized studies suggest that anaemia is a risk factor for mortality in hip fracture patients. We assessed the correlation between haemoglobin at admission with 30-days mortality following a hip fracture in a large-scale study.
Method: From January 1996 to November 2013, all hip fracture patients (> 60 yrs of age) admitted to Bispebjerg Hospital, Denmark, were identified using a dedicated local fracture database. We excluded conservatively treated patients and patients who died preoperatively. Prior co-morbid conditions were identified through linkage to the National Hospital Discharge Register.
Results: We identified 7755 consecutive hip fracture patients. Of these, 7647 (98.6%) had a haemoglobin measurement on admission and were thus eligible for further analysis. Mean haemoglobin for patients alive at 30 days was 7.6 (SD 1.0), and for deceased patients 7.4 (SD 1.1), p < 0.0001. Mean age was 82.5 years (SD 8.5) and 76.4% of the population were female ($N_{females}$ = 5845). The 30-days mortality in the 1st, 2nd, 3rd, and 4th quartiles of haemoglobin was 13.2%, 10.1%, 9.2% and 8.9%, respectively (p < 0.0001). In the multivariate analysis, the hazard ratio (HR) with 95% confidence interval (CI) for 30-days mortality in anaemic patients (< 7.3 mmol/L for females and < 8.5 mmol/L for males. N_{anemic} = 3364) was 1.70 CI [1.48-1.96], p < 0.0001. Adjusting for age, gender and co-morbidities (Charlson Score) slightly attenuated the risk estimate (HR 1.23 CI [1.05-1.42], p = 0.0008)

Conclusion: This analysis indicates that anaemia at admission is significantly associated with 30-days mortality in hip fracture patients even after adjusting for comorbid diseases. This information might be used to identify patients with a higher risk of death using inexpensive and easily interpreted haemoglobin tests and thus results in intensified care in order to reduce the excess mortality.
Disclosure: The authors declared no competing interests.

P316
MicroRNA Expression in Osteoblasts is Influenced By Oestrogens and Oxidative Stress
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Oestrogen deficiency and oxidative stress are the main factors responsible for postmenopausal and age-related bone loss. In addition, epigenetic mechanisms and in particular miRNAs, are becoming increasingly recognised as significant molecular regulators of bone cells. Our aim was to investigate how oestrogens and oxidative stress alone or in combination influence the expression of miRNAs in osteoblasts. An osteoblast cell line HOS was transfected with a plasmid containing oestrogen receptor α and exposed to 17β-estradiol (E2) and/or hydrogen peroxide. Western blotting was used to confirm the successful transfection and the *AOX1* gene expression was measured in order to verify the establishment of oxidative stress. Nanostring nCounter technology was used to obtain the expression profile of 800 miRNAs. Our results revealed only a slight influence of E2 on miRNA expression, while the impact of hydrogen peroxide was significantly larger. We observed downregulation of miR-338-3p after E2 treatment. Hydrogen peroxide on the other hand positively affected expression of miR-132-3p and miR-630 among other, while downregulating miR-133 and miR-214-3p among other. miR-133, miR-214-3p and miR-338-3p have already been shown to have important roles in osteoblast biology. Of note, E2 exhibited no protective effect when co-treated with hydrogen peroxide. Even though the investigated stimuli and miRNA have been studied separately in osteoblasts before, our results are the first to show that oxidative stress exhibits a significant impact on miRNA expression in osteoblasts.
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P317
Endothelial Dysfunction in Chronic Obstructive Pulmonary Disease and Osteopenic Syndrome
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Background: The aim was to investigate the role of biomarkers of endothelial dysfunction in formation of osteoporosis in the patients with chronic obstructive pulmonary disease (COPD).
Methods: Soluble E-selectin, endotheline 1 (ET-1), vitamin D, vascular endothelial growth factor (VEGF), metalloproteinase-9 (MMP-9), vascular cell adhesion molecule (VCAM-1), osteoprotegerin (OPG), the receptor activator of nuclear factor-kB ligand (RANKL) and bone biomarkers were determined in 63 patients with COPD. BMD was measured by DEXA at the lumbar spine (LS) and left femur neck (FN).
Results: Circulating bone formation markers (procollagen type 1 amino-terminal propeptide P1NP), N-terminal mid-molecule fragment osteocalcin (N-MID OC) and bone specific alkaline phosphatase was lower in COPD than in the controls. Type 1 collagen C-telopeptide (CTx-bone resorption marker) was higher in lung group and was inversely related to FN (r=-0,43, p<0,05) and had a direct relationship with P1NP (r=0,63, p<0,01). The E-selectin, endotheline 1 (ET-1), TNF-a, IL-6, VEGF, RANKL, MMP-9 and VCAM-1 were higher; Vitamin D, OPG were low in lung pathology than in controls. Compared with the lung group with osteopenia, levels of E-selectin, TNF-a, ET-1, MMP-9 and VEGF were the highest in COPD with osteoporosis. There was positive correlation between vitamin D, OPG (r=0,51, p<0,001; r=0,57, p<0,001) and negative one between TNF-a (r=-0,44, p<0,01), MMP-9 (r=-0,46, p<0,01) and BMD in FN and LS ; inverse correlation between ET-1 (r=-0.56, p<0,01), VEGF (r=-0.64, p<0,001) in L2-L4 only ; negative correlation of VCAM-1 and TNF-a with BMD in FN only. OPG was correlated with N-MID OC (r=0,53, p<0,001), RANKL (r=-0,44, p<0,01) and TNF-a in lung patients. No correlations were found between VCAM-1, IL-6 and markers of bone metabolism. ET-1, MMP-9 and VEGF significantly positively correlated with parameters of bone resorption and negatively associated with P1NP and N-MID OC.
Conclusion: A significant association between parameters of bone metabolism and endothelial dysfunction markers in COPD patients with osteoporosis, which suggests possible role of endothelial dysfunction in the increasing of bone loss in COPD.
Disclosure: The authors declared no competing interests.

P318
Vitamin D status and Bone Health: a Population-Based Study of Japanese School Children
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Background: A number of studies have investigated the relationship between 25-hydroxyvitamin D (25-OH-D) and bone mineral density (BMD) during childhood. However, there is considerable variation in study subjects, geographic location, season in which the study is conducted, and confounding factors considered. Consequently, reported relationships between 25-OH-D and BMD have been somewhat inconsistent. There remains a substantial shortage of information concerning the Japanese child population. In the present study, we investigated the relationship between serum

25-OH-D concentration and whole body BMD among Japanese children.
Methods: The source population was all fifth-grade students (521 children) attending two public elementary schools in Hamamatsu, Japan. Among these, we obtained cross-sectional data from 401 children (mean age, 11.2 years) in November and December. Whole body bone mineral content (BMC) and whole body BMD were measured using a single dual-energy X-ray absorptiometry scanner. In addition, a non-fasting blood specimen was collected from each subject. Serum 25-OH-D concentration was measured by a radioimmunoassay. This study was approved by the Ethics Committee of our university.
Results: Boys showed positive relationships between serum 25-OH-D concentration and whole body BMD and BMC after adjusting for confounding factors, such as height, weight, and pubic hair appearance (BMD, $\beta = 0.20$; BMC, $\beta = 0.09$; $P < 0.05$ for each value). Girls showed a positive relationship between serum 25-OH-D concentration and whole body BMD after adjusting for confounding factors ($\beta = 0.13$, $P < 0.05$).
Conclusion: A high serum vitamin D concentration was associated with good bone health in a population of Japanese school children.
Disclosure: The authors declared no competing interests. This work was supported by Grants-in-Aid for Scientific Research (#21657068 and #22370092) from the Japan Society for the Promotion of Science.

P319

Relationship between Osteoporosis and Hypertension with Low Calcium and High Sodium Dietary Intake in Korean Population (KHANES IV-V, 2008-2011)

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Osteoporosis and hypertension are examples of major public health problems with significant morbidity and mortality. Coexistence of osteoporosis and hypertension, which are considered to be distinct diseases, has been observed for more than a century and suggested that they might be genetically and aetiologically related. Daily intake of calcium and sodium is known to be associated with osteoporosis and hypertension, respectively, and low calcium and high sodium dietary intake is characteristics of the Korean population. This study aims to find the role of low calcium and high sodium dietary intake in a Korean population in association with osteoporosis and hypertension. The data from Korea National Health and Nutrition Examination Survey 2008-2011 were included in this study. Osteoporosis was diagnosed by dual energy X-ray absorptiometry and hypertension was diagnosed by blood pressure data or the use of antihypertensive medication. Daily calcium and sodium intake was calculated using 24-hour dietary recall questionnaire. The odds of osteoporosis and hypertension were calculated for quartiles of daily calcium and sodium intake by logistic regression analysis. Men with hypertension had higher prevalence of osteoporosis (6.4% vs. 3.5%; $p = 0.002$), and vice versa (22.6% vs. 13.6%; $p = 0.002$). Women with hypertension also had high coexistence of osteoporosis (32.2% vs. 11.5%; $p < 0.001$), and vice versa (19.6% vs. 6.2%; $p < 0.001$). Analysis according to quartiles of calcium and sodium intake showed that only calcium was significantly associated with both diseases. We concluded that osteoporosis and hypertension are associated in Korean population, and a low dietary calcium intake could be associated with both diseases, suggesting a possible pathogenic linkage.
Disclosure: The authors declared no competing interests.

P320

Bone Mass in Down Syndrome

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Background: A relationship between osteoporosis and Down syndrome (DS) has been suggested. The causal mechanisms are unclear, but they could involve differences in the skeletal size.
Methods: Seventy-six patients with DS and 77 controls were included in the study. Spine and hip BMD values were assessed by dual-energy X-ray absorptiometry. Volumetric BMD was estimated by previously published formulas[1] Serum 25OHD and iPTH levels were measured.
Results: The average age was 33 (18-64); 53% were men. Weight and height were lower in DS group (weight: 60 vs. 69 Kg; $p<0.001$ and height: 151 vs. 169 cm; $p< 0.001$), but BMI was higher in DS (26.5 vs. 24.1 kg/m^2; $p<0.001$). Baseline 25OHD and iPTH levels were similar in both groups (22.7 [7.9] vs. 24.4 [9.6) ng/ml, $p=0.25$; and 24 [10] vs. 26 (14) pg/ml, $p=0.32$, respectively). Similarly, the prevalence of hypovitaminosis D (25OHD<20 ng/ml) was similar in both groups (39% vs. 35%). Areal hip and spine BMD values were lower in people with DS. However, the estimated volumetric BMD was similar in both groups. (See Table 1.)
Conclusion: Areal BMD is reduced in Down syndrome, but it seems to be related to the smaller body and skeletal size. In fact, the estimated volumetric BMD is similar in patients with Down syndrome and in control individuals.
Disclosure: The authors declared no competing interests.

Reference

1. Guijarro *et al.* (2008) J Intellect Disab Res.

Table 1 [P320]

		DS	CONTROLS	*p*
Lumbar spine	DMO (g/cm^2)	0.903 (0.117)	0.997 (0.121)	< 0.05
	DMOv (g/cm^3)	0.244 (0.030)	0.255 (0.037)	0.061
Femoral neck	DMO (g/cm^2)	0.761 (0.099)	0.838 (0.091)	< 0.05
	DMOv (g/cm^3)	0.325 (0.068)	0.309 (0.043)	0.104

P321

Metabolic Syndrome Components are Differentially Associated with Bone Density and Turnover in Middle-Aged and Elderly European Men

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Background: Metabolic syndrome (MetS) and its components (waist circumference, triglycerides, HDL, blood pressure, fasting glucose) have been associated with bone health, but studies in men are moderate-sized and unequivocal.
Methods: Men 40-80 years were recruited to eight centres across Europe. Medications, smoking, drinking, physical activity, weight, height, blood pressure and walking speed were recorded. We measured glucose, insulin, HDL/LDL cholesterol, triglycerides; SHBG, IGF1, 25(OH)D, PTH, leptin by immunoassay; and sex steroids and 1,25$(OH)_2$-D by mass spectrometry. MetS was defined by 2009 NCEP-ATPIII criteria. Outcomes included bone turnover markers (BTMs) and heel quantitative ultrasound [broadband ultrasound attenuation (BUA), speed of sound (SOS) and quantitative ultrasound index (QUI)].
Results: After excluding men on glucocorticoids, hormonal/bone drugs or those with missing data, 3068 were analysed. Men with MetS men (31.7%) were older, more ex-smokers, and had lower alcohol intake and physical performance. They also had lower total and free testosterone, SHBG, 25(OH)D and 1,25$(OH)_2$-D, IGF1, and higher FSH, bioavailable and free E2, leptin, CRP and HOMA-IR. In linear regression analyses (unadjusted; adjusted for age, centre, smoking, alcohol; and additionally adjusted for BMI), MetS status was inversely associated with -cTX, P1NP and osteocalcin ($P<0.0001$). However, only triglycerides and glucose were associated independently of other MetS components and BMI. BMI was not associated with BTMs independent of MetS components. Measures of insulin resistance (HOMA-IR/QUICKI, fasting insulin, HOMA-S and SHBG) consistently attenuated the association between MetS and BTMs, but hormones or physical activity did not. MetS was positively associated with BUA and QUI, but not following BMI adjustment. BMI was positively associated with BUA, SOS and QUI, independent of MetS components. Fasting glucose, insulin and HOMA-IR were negatively associated with BUA following BMI adjustment.
Conclusion: Men with MetS have lower bone turnover and higher bone density, which are differentially linked to insulin resistance and obesity, respectively.
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P322

Experimental Myocardial Infarction does not Induce Osteopenia

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Background: Fracture risk is significantly increased in patients with myocardial infarction (MI) and heart failure but the causal relationship of this association is unknown. Using an experimental model of MI in mice and rats, we aimed to investigate whether MI *per se* induces osteoporosis.
Methods: MI was induced in adult 5-month-old C57BL/6 mice by permanent ligation of the left anterior descendent (LAD) coronary artery. Ischaemia/reperfusion injury was induced in 4- and 9-month-old Fischer-344 rats by LAD ligation for 30 minutes followed by reperfusion. Cardiac function was assessed by echocardiography. Bone mineral density (BMD) was analysed using quantitative peripheral computed tomography (pQCT) 4- and 9 weeks after MI in mice, and 4 weeks after MI in rats. Bone resorption was analysed by urinary deoxypyridinoline (DPD) excretion.
Results: In mice, MI reduced fractioning shortening (FS) from 31.32±0.56% to 22.11±1.8%. Four weeks after MI, total BMD of the femoral metaphysis (423.7±7.4mg/cm^3) and the femoral shaft (591.6±9.7mg/cm^3) did not significantly differ from sham (metaphysis: 427.5±8.7mg/cm^3, shaft: 612.7±9.1 mg/cm^3). Vertebral BMD was also unchanged. Similarly, femoral and vertebral BMD was unaffected 9 weeks after MI. In addition, bone resorption remained unchanged, 4 weeks after MI. To exclude a species specific skeletal response to cardiac ischemia, we additionally employed a rat I/R injury MI model. MI decreased FS by 9%, but BMD and bone resorption in young rats (4-month-old) remained largely unchanged as measured at the tibial metaphysis, tibial shaft and first lumbar vertebra, 4 weeks after ischaemia (BMD at tibial metaphysis 581.1±9.2mg/cm^3 in sham vs. 602.9±8.9mg/cm^3 in MI). Finally, BMD was also not affected by cardiac ischemia in aged 9-month-old rats.
Conclusion: Our data suggest that experimental myocardial infarction *per se* has no direct effect on bone resorption or bone mineral density. Further research is needed to explain the increased bone fragility in cardiovascular patients.
Disclosure: The authors declared no competing interests.

P323

The Relationship between Bone and Muscle and its Affecting Factors in a Korean Population

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Background: Muscle mass is known to be related with bone mineral density. However there were few data for affecting factor to this relationship. In this study, we investigated the affecting factor to the relationship between bone and muscle.

Methods: The data from a population-based survey, namely, The Korea National Health and Nutrition Examination Survey (KNHANES) IV (08-09) & V(10) (18,007 subjects), were analysed. We studied the relating factor (anthropometric data and insulin resistance) to bone mineral density (BMD)/[appendicular lean mass(ALM)/(height)^2].

Result: With increasing age, BMD/ALM(ht)2 was increasing in men (B=0.126, p<0.001) but deceasing in women (B= -0.408, p<0.001). After adjusting age, fat mass (men: B=-0.186, p<0.001, women: B=-0.162, p<0.001) and waist circumference (men: B=-0.186, p<0.001, women: B=-0.162, p<0.001), fasting insulin level (men: B=-0.108, p=0.002, women: B=-0.088, p=0.016) was negatively related with BMD/ALM(ht)2. And insulin resistance (HOMA-IR) was also negatively related with BMD/ALM(ht)2 (men: B=-0.115, p<0.001, women: B=-0.121, p<0.001). HDL cholesterol level had positive relation with BMD/ALM(ht)2 (men: B=0.081, p<0.001, women: B=0.115, p<0.001).

Conclusion: In this study, bone and muscle relation was differing between men and women. And obesity and insulin resistance may be weakening the relationship between bone and muscle.

Disclosure: The authors declared no competing interests.

P324

Factors Associated with the Development of Osteoporosis after Recent Spinal Cord Injury: a 12-Month Follow-Up Study

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Background: Spinal cord injury (SCI) is associated with a marked bone loss short-term after injury and a consequent increased risk of osteoporosis and fractures. We recently observed the development of osteoporosis in nearly 50% of SCI patients one year after injury. Therefore, the aim of this study was to evaluate the factors associated with osteoporosis development in these patients.

Methods: We included patients with complete recent SCI (<6 months) evaluating bone turnover markers (P1NP, bone ALP and sCTx), 25-OH vitamin D levels and lumbar and femoral BMD at baseline, 6 and 12 months after SCI. The risk factors for osteoporosis analysed included: age, gender, BMI, toxic habits, bone turnover markers, 25-OH vitamin D levels, lumbar and femoral BMD, level, severity and type of SCI and days-since-injury. Osteoporosis was defined according to WHO criteria.

Results: 29/42 patients completed the 12-month follow-up. 52% developed osteoporosis during follow-up. Patients who developed osteoporosis had lower BMD values at femur and lumbar spine and higher bone turnover markers values (P1NP and bone ALP) at baseline. On multivariate analysis, the principal factors related to osteoporosis development were: total femur BMD <1 gr/cm^2 (RR, 3.61; 95%CI, 1.30-10.06, p=0.002) and lumbar BMD <1.2 gr/cm^2 at baseline (0.97 probability of osteoporosis with both parameters under these values). Increased risk for osteoporosis was also associated with increased baseline values of bone ALP (>14ng/mL) (RR, 2.40; 95%CI, 1.10-5.23, p=0.041) and P1NP (>140ng/mL) (RR, 3.08; 95%CI, 1.10-8.57, p=0.017). Conversely, age, BMI, type and time-since-SCI or 25-OHD levels were not related to increased risk of osteoporosis over 12 months.

Conclusions: The evaluation of BMD at the lumbar spine and femur short-term after SCI constitutes the principal factor for predicting the development of osteoporosis during the first year after SCI. These data indicate the need to evaluate and treat these patients shortly after injury.

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P325

Effect of Type 2 Diabetes Mellitus on Bone Cells, Turnover and Density

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Type 2 diabetes mellitus (T2DM) increased the risk of fragility fractures, even if it is often associated with an increase in bone density. T2DM could impact bone through several mechanisms. We evaluated the impact of diabetes on bone by measuring bone turnover, bone cells precursors and cytokines in 50 T2DM patients and 100 age and BMI matched controls. In order to evaluate the effect of T2DM on bone density (BMD), quality (TBS) and the incidence of fragility fractures we measured these parameters by DXA, in T2DM BMD was significantly higher at lumbar spine, TBS was inversely correlated with glycaemic control measured by HbA1C (R=0.35, p<0.0001), but we found no increase in the incidence of vertebral and non-vertebral fractures. Bone turnover was reduced in T2DM patients whereas osteoclast precursors in peripheral blood were significantly increased and osteoblast precursors decreased. Amongst cytokines involved in the control of bone turnover RANKL was decreased in T2DM patients, whereas there were no differences in the levels of OPG, consequently the RANKL/OPG ratio was increased in T2DM. Levels of SOST and DKK-1 were not significantly influenced by T2DM. Muscular strength and balance were significantly reduced in T2DM patients as respect to controls, with particular regard to the hand grip test and the Tinetti balance test, whereas the Timed up and

Go test was not significantly different between T2DM and control. In conclusion, we observed a decreased turnover in T2DM even though osteoclast precursors were increased in peripheral blood. This result may be due to an increase in immature osteoclasts that are not recruited in bone, in fact RANKL was decreased in T2DM as respect to controls. We did not found significant difference in the incidence of fragility fracture in T2DM, this could be due to the use of controls matched for obesity and age; moreover we found no differences in TBS in diabetic patients, interestingly the bone quality seems to be worsened by the scarce glycaemic control. Muscular force and balance was impaired in T2DM, this could influence the risk of non-vertebral fragility fractures, anyway the low sample does not allow us to investigate this type of fracture.
Disclosure: The authors declared no competing interests.

P326
The Incidence of New Osteoporotic Fracture is Related to Vitamin K Insufficiency by Measuring ucOC in Postmenopausal Japanese Women
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Background: The high serum undercarboxylated osteocalcin (ucOC) value is a risk factor for proximal femoral fractures independent of the BMD. The vitamin K insufficiency is indicated of high ucOC value. Natto, which is one of the food to eat in breakfast well in Japan, is a major source of vitamin K. The purpose of present study is to determine whether the incidence of new osteoporotic fractures is associated with vitamin K insufficiency and with the ucOC value, a surrogate marker of vitamin K insufficiency in bone in postmenopausal Japanese women.
Methods: We conducted a survey in 289 postmenopausal women who were inpatients or outpatients. Age, vitamin K intake (frequency of natto intake per week), ucOC value, urinary cross-linked N-telopeptides of type I collagen (u-NTX), BMD (lumbar, femoral neck, total hip), the presence or absence of new osteoporotic fractures, and use of osteoporosis medications (antiresorptive drugs, other drugs, none) were surveyed.
Results: The mean age of the subjects was 74.6±9.6 years. As many as 207 (72%) of the 289 women reported low natto intake ("once a week or less" or "none") and thus low intake of vitamin K. Low natto intake was associated with significantly higher ucOC values. The incidence of new osteoporotic fractures increased with decreasing natto intake. Further analysis revealed a higher incidence of new osteoporotic fractures in the high-ucOC subgroup. A multivariate logistic regression using the presence or absence of new osteoporotic fractures as an objective variable revealed that age, total hip BMD, and ucOC value were independently associated with the incidence of new osteoporotic fractures.
Conclusions: High ucOC values were demonstrated to be associated with insufficient intake of vitamin K and with the appearance of new osteoporotic fractures.
Disclosure: The authors declared no competing interests.

P327
Co-Expression of Adipogenic and Osteoblastic Proteins in MSC-Derived Osteoblasts Following Co-Culture with MSC-Derived Adipocytes
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In osteoporosis, bone loss is accompanied by an increase of adiposity in the marrow. A dialogue between adipocytes and osteoblasts is one of the ways occurring in the competition between Mesenchymal Stem Cells (MSCs) lineage commitments, supporting adipocyte differentiation at the expense of osteoblast differentiation. Using an *in vitro* coculture model based on human primary MSCs, we have previously shown that MSC-derived adipocytes are capable of inducing MSC-derived osteoblasts to differentiate towards an adipocyte-like phenotype. Indeed, upon coculture, MSC-derived osteoblasts showed appearance of adipocyte and decrease of late osteogenic mRNA markers. To confirm the co-localisation of adipogenic and osteoblastic proteins on a single cell level, we performed double immunofluorescence microscopic analyses. The results clearly showed an expression of osteoblast specific protein osteocalcin with adipogenic marker leptin in osteoblasts incubated with adipocytes conditioned medium, while in control osteoblasts expression of osteocalcin could only be observed. So, we provide evidence that MSC-derived osteoblasts can transdifferentiate into another lineage under the influence of secreted products release by MSC-derived adipocytes. We further aim to elucidate the mechanisms implicated in this phenotypic conversion, this knowledge being a prerequisite to target the competition between osteoblasts and adipocytes and to conceive a new approach for treatment of osteoporosis.
Disclosure: The authors declared no competing interests.

P328
Mortality in Patients with Hip Fracture Within Two Years After the Trauma
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Background: The aim was to analyse the value of mortality, as well as factors affecting the survival of the patients aged ≥50 years within 2 years after the trauma.
Methods: A prospective study includes all the inhabitants of the city of Yaroslavl 50 years of age and older who had low-energy hip fractures from 01.09.2010 to 31.08.2011.

Results: The study included 446 patients: 334 (74.88%) women and 112 (25.12%) men (average age was 76,83±10,32 yrs). The overall mortality rate during within 2 years it was 43.9%. In 24 months after hip fracture the died patients were significantly older than the survivors (78,71±9,17 yrs vs 73,41±10,39 yrs, p=0.02). The factors which significantly affected mortality rates (p<0,05) were: a history of coronary heart disease, psychosis during hospitalisation, the index over the 3rd degree on the scale of anesthetic risk (ASA); cognitive impairment (MMSE scale corresponding as dementia or encephalopathy of II-III degree), the level of physical activity on a scale of Katz Activity of Daily Living ≤"F", walking <30 minutes a day after the injury, value of creatinine >100 mmol/l at admission. Significant differences between men and women were not received either. The influence of age proved to be true in groups aged ≥60 and ≥80 and older. Among operated patients mortality was significantly less (27.59%) than among patients who were treated conservatively (53,54%).
Conclusions: The mortality among patients with hip fracture within 2 years was 43.9%. Higher rates of mortality were significantly associated with older age, presence of coronary heart disease, anaemia, azotemia, cognitive impairment, low level of physical activity after the fracture, and absence of surgical treatment.
Disclosure: The authors declared no competing interests.

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The Incidence and Mortality After Hip Fracture in Korea

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Background: We determined the incidence of hip fracture and subsequent mortality in Korea using national wide data from the national health insurance service from 2008 to 2012.
Methods: This study was performed on the patient population, aged 50-year older who underwent surgical procedures because of hip fracture (ICD 10; S720, S721). All patients were followed using patient identification code to identify deaths.
Results: Crude incidence of hip fracture increased from 221.4/100,000 to 299.4/100,000 in women and from 104.4/100,000 in 2008 to 131.2/100,000 in 2012 in men, respectively. Crude mortality within 12 months after hip fracture showed a similar trend (16.7% in 2008 and 14.9% in 2012). The standard mortality ratio at 1 year after hip fracture decreased from 3.2 in 2008 to 2.8 in 2012 and trend of mortality rate in women were more significant than in men (-4.1% in men and -14.3% in women).
Conclusions: The increasing incidence and the high mortality after hip fracture are still serious public health problems in recent years and a public health programme should be more active and systematic to decrease hip fractures in the future.
Disclosure: The authors declared no competing interests.

P330

Low Bone Mineral Density and Comorbidities in Patients with Rheumatoid Arthritis

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It's known, the rate and extent of bone loss in RA can be influenced by various factors associated both with the RA and other diseases. The purpose was to study influence of common comorbidities on prevalence of osteoporosis (OP) in patients with RA in clinical practice. Through of the national program we analysed data of Dual-energy X-ray absorptiometry (DXA) in lumbar spine, femoral neck and forearm on 691 patients. Two groups of patients were formed: with and without OP 196 (28%) and 495 (72%), respectively. In both groups, the number of women prevailed, the average age of 60,9±2,8 in group 1, 56,1±2,1 in group 2[p>0,05]. 81% and 69% women had menopause, respectively [p<0,05]. The average duration of RA was 17±2,1 years, 15,4±7,1, respectively [p <0,05]. 51% and 41% of patients had one or more comorbidities, respectively [p<0,05]. To select the high risk group of OP and fractures among patients with RA in clinical practice should keep in mind such factors as age, menopause, duration of RA and comorbidities. (See Table 1)
Disclosure: The authors declared no competing interests.

Table 1 [P330]

Group with OP	Group without OP
22% EP[1] (87% TD[2], 11% diabetes type 2)	15% EP[1] (79% TD[2], 19% diabetes type 2)
66% CVD[3] (64% H[4], 26% CHD[5], 2% [6]MI, 8% stroke)	49% CVD[3] (77% of H[4], 19% CHD[5], 2% [6]MI, 2% stroke)
14% RP[7] (57% COPD[8], 14% asthma, 4% tuberculosis, 4% sarcoidosis, 21% PF[9])	14% RP[7] (80% COPD[8], 12% asthma, 4% tuberculosis, 4% PF[9])
27% GIP[10](38% SU/DU[11], 36% CG[12], 2% cirrhosis, 2% GERD[13], 5% VH[14], 2% GSD[15])	27% GIP[10] (35% SU/DU[11], 45% CG[12], 1% GERD[13], 1% VH[14])

[1]endocrinologocal pathology, [2]thyroid disease, [3]cardiovascular disease, [4]hypertension, [5] coronary heart disease, [6]myocardial infarction, [7]respiratory pathology, [8]chronic obstructive pulmonary disease, [9]pulmonary fibrosis, [10]gastrointestinal pathology, [11]stomach/duodenal ulcer, [12]chronic gastritis, [13]gastroesophageal-reflux disease, [14]viral hepatitis, [15]gallstone disease.

P331

Biochemical Predictors of Low Bone Mineral Density and Fracture Susceptibility in Maltese Postmenopausal Women

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Background: Osteoporosis and fracture risk are polygenic conditions which result from an interplay of genetic, biochemical and environmental factors. A number of biochemical markers including serum calcium, total alkaline phosphatase (ALP) and albumin, were analysed in relation to bone mineral density (BMD) and different types of low-trauma fractures in Maltese postmenopausal women. Levels were also correlated with a number of clinical risk factors including physical activity and years since menopause (YSM).
Methods: An age-matched case-control study of 1045 women was performed. Women who suffered low-trauma fractures were classified as cases whereas those without a fracture history were included as controls subdivided into normal, osteopenic or osteoporotic according to their BMD status. Blood specimens were collected following good standard practice and within 18 hours of fracture in the case of fresh-trauma fractures. Biochemical testing was performed using spectrophotometric analysis.
Results: Serum calcium, and to a lower extent ALP levels, were correlated with BMD levels at the femoral neck, FN (calcium rho: 0.111, $P<0.01$, ALP rho: 0.089, $P<0.05$). Fracture cases had the lowest levels of serum calcium, ALP and albumin relative to all other control groups ($P<0.05$), which significantly decreased with increasing age ($P<0.05$), possibly contributing to an increased fracture risk due to reduced intestinal absorption, malnutrition and depleted protein levels, as well as a lower overall well-being. Levels were lowest in women who sustained a hip fracture and in those who sustained more than one fracture ($P<0.05$). YSM was correlated with lower calcium levels in fracture cases (rho: -0.229, $P<0.01$). Low physical activity was associated with low BMD at the lumbar spine (LS) and FN ($P<0.00$), and with lower concentrations of serum calcium, ALP and albumin ($P<0.05$).
Conclusion: Results suggest that measurements of serum calcium, ALP and albumin levels could be indicative of frailty and low BMD.
Disclosure: The authors declared no competing interests.

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Vitamin D Deficiency in Patients with Psoriatic Arthritis and its Role in Disease Activity

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Background: Vitamin D is crucial in calcium homeostasis, playing a role in the modulation of the immune system. The objectives were to assess BMD, bone turnover markers and 25OHD levels in psoriatic arthritis (PsA) patients; and to investigate the relationship between 25OHD and disease activity.
Methods: Fifty patients were recruited. Patients with axial involvement were excluded. Calcium, phosphorus, 25OHD, PTH, P1NP and βCTX were measured as bone turnover markers. BMD was measured at the lumbar spine and hip by DXA. Disease activity was assessed using DAS-28, BASDAI, CPR and ESR levels; HAQ for functional impairment.
Results: Twelve premenopausal women, 22 postmenopausal women and 16 men were included. The clinical forms of PsA were: 32% oligoarticular and 54% poliarticular. Mean disease duration was 111 ± 108 months, 25OHD levels was 28,91 ± 13,3 ng/dl, DAS 28 1,61 ± 0,70 and BASDAI 3,24 ± 1.99. Sixteen patients (32%) presented insufficient 25OHD levels (< 30 ng/ml) and 22% showed deficiency(< 20 ng/ml). Frequency of osteoporosis was 14% and osteopenia 49%, being higher in postmenopausal women (75%) rather than premenopausal (25%) or men (29.4%). P1NP 38,9 (14-72) ng/ml and βCTX 341,2 (142-866) pg/ml had normal values. Six fractures were registered. Mean values of ESR, CPR and HAQ were 10,9 ±11,41 mm/h, 5,38 ± 0,86 mg/L and 0,33 ± 0,48 respectively, in patients with normal vitamin D levels; whereas patients with low vitamin D levels presented higher values (ESR, CPR and HAQ mean values of 12,1 ± 9,82 mm/h, 6,48 ± 5,38 mg/L and 0,37 ± 0,56). Our results are not statistically significant due to low sample population.
Conclusions: High prevalence of 25OHD insufficiency was found. 63% had decreased bone mass. An inverse correlation between 25OHD levels, disease activity and functionality is shown. There is a relationship between high disease activity in PsA and 25OHD metabolism and increased bone resorption.
Disclosure: The authors declared no competing interests.

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Determination of the Relationship between Serum Levels of Vitamin D, FSH, or Oestradiol and Fracture Risk in Postmenopausal Taiwanese

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Background: Serum levels of vitamin D, FSH, and oestradiol are associated with bone health. Vitamin D has numerous biologic effects such as enhancing calcium and phosphate absorption, production of antimicrobial peptides, anti-inflammatory activities, and development of regulatory T-cells. Insufficiency and deficiency of vitamin D has been linked

to increased risk of cardiovascular diseases, infectious diseases, muscle-skeletal diseases, and cancer. The aim of this study was to determine the relationship between vitamin D insufficiency and the 10-year fracture risk compared with that of abnormal serum FSH or oestradiol levels in postmenopausal women in Taiwan.
Methods: Serum vitamin D3 [25(OH)D3], FSH, and oestradiol levels of 610 postmenopausal women were measured. These women were recruited from August 2010 to October 2013 when they visited the menopause clinic. Only those without major surgery or chronic diseases were included. The following parameters were also obtained: blood pressure, pulse rate, body temperature, body weight, body height hot flush, sweating, sleep disturbance, headache, previous fracture, living habits, alcohol consumption, and smoking.
Results: Six hundred and ten Taiwanese women in postmenopausal status with mean age 54.8 and mean years from menopause of 5 years were collected from Aug. 2010 to Oct. 2013. Those serum FSH were all higher than 30 unit/ml and oestradiol were low as all lower than 30 pg/ml. There were 515 women (84%) whose serum level of vitamin D was less than 30 ng/ml. Twenty-seven postmenopausal women had higher major fracture risk, which was counted by FRAX and higher than 10 % and 35 postmenopausal women had higher hip fracture risk which was larger than 3 % counted by FRAX without BMD data. The correlations between fracture risk and serum Vit D or FSH or oestradiol were calculated separately by Pearson analysis.
Conclusions: High serum FSH levels were found to have a higher correlation than vitamin D insufficiency with 10-year fracture risk in postmenopausal women in Taiwan.
Disclosure: The authors declared no competing interests. This work was supported: Grants: CMRPG891191-3 and CMRPG891171-3.

P334
Bone Mass and Body Composition in COPD Patients
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Chronic obstructive pulmonary disease (COPD) is a multisystemic disorder, affecting also the musculoskeletal system and body composition. We investigated in a group of 93 COPD patients (69 men, age 66.3+/-9.0 years; 24 women, age 61.9+/-7.2 years) associations of bone mineral density (BMD), body composition (BMI – body mass index, FMI - fat mass index, FFMI - fat-free mass index), severity of COPD (Gold classification) and vitamin D status. Standard clinical (DEXA) and laboratory methods were used. Osteoporosis was found on at least one measured site (lumbar spine, femoral neck or trochanter) in 23% male and 21% female patients. Patient groups with osteoporosis or normal BMD did not differ regarding COPD severity, 25-OH D concentrations or age. Osteoporosis in both sexes was associated with significantly lower BMI ($p<0.001$) and FMI ($p<0.002$), and in men with lower FFMI ($p<0.003$). BMI was on average normal in patients with osteoporosis and increased in patients with normal BMD in both sexes. Patients of both sexes with normal BMD had increased FMI. Male osteoporosis patients had normal or increased FMI and female osteoporosis patients had normal FMI. FFMI was increased in all male patients. In women with osteoporosis, FFMI was mostly decreased and in those with normal BMD was normal or decreased. All female patients had hypovitaminosis D (<50 nmol/L). Hypovitaminosis D was found in most male patients; in 14/16 patients with osteoporosis and in 47/53 patients with normal BMD. In conclusion, osteoporosis was observed in more than 20% of COPD patients and hypovitaminosis D in most patients. In male COPD patients with osteoporosis muscle mass was preserved and fatty tissue normal or increased. In female COPD patients with osteoporosis both muscle mass and fatty tissue were decreased.
Disclosure: The authors declared no competing interests.

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Long-Term Effect of Phytoestrogens on the Ovariectomy Response in the Cynomolgus Monkey Model of Osteoporosis
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Phytoestrogens (PEs) are naturally occurring plant polyphenols with oestrogenic activity. Soy protein-free diet excludes alfalfa and soybean meal, minimising PE content. This study evaluated long-term effects of 2056 Soy Protein-Free (SF) diet on bone turnover, mass and strength over 18 months (M) in aged OVX Mauritius monkeys. Animals fed SF diet were compared with historical data[1] obtained from animals fed standard diet (SD). Compared with sham, biomarkers for OVX animals showed greater increases in those fed SF diet relative to SD. At 6 and 18M: on SF diet, osteocalcin increased 19% and 22%, serum CTx 16% and 12%, Bone-ALP 58% and 62%, relative to SD. Greater increases in biomarkers were associated with more consistent and greater decreases in bone mass. Relative to sham at 6 and 18 M, for animals fed SF diet, DXA BMD decreased 9% and 10% at lumbar spine and 8.7% and 9% at proximal femur, respectively. At 18 M, animals fed SD showed decreases of 4.8% at lumbar spine and 5.2% at proximal femur. On SF diet tibia trabecular pQCT BMD decreases were 16.5% and 24% at 6 and 18M, with decreases in cortical BMD at the tibial diaphysis of 5.4% and 5.3%. These decreases were more marked at 18 M compared with SD animals, which showed decreases of 11% for trabecular BMD and 3.7% for cortical BMD. Bone strength parameters were similar for animals on either diet. These data suggest dietary PEs may influence the OVX response in aged monkeys as evidenced by greater increases in bone turnover and loss of bone mass on SF diet allowing better discrimination when evaluating potential anti-osteoporosis drugs.
Disclosure: The authors declared no competing interests.

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Body Composition and Bone Health in Patients with Type 2 Diabetes Mellitus

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Background: Patients with type 2 diabetes (T2DM) have an increased risk of hip and vertebral fractures compared to non-T2DM subjects. The bone mineral density (BMD) does not reflect the impaired bone quality in T2DM patients. The aim of the present study was to investigate the relationship between BMD and body composition as well as bone and calcium metabolism parameters.
Methods: Total body fat (FM) and lean mass (LM), total body bone mineral content (TBBMC) as well as total body (TB), hip (total femur and femoral neck), lumbar spine (LS) and radius BMD were measured with DXA.
Results: In our cohort of 140 patients with T2DM 31% had normal BMD, 47% osteopenia, 22% osteoporosis. LM was positively associated with TBBMC and TB, hip, LS as well as radius BMD ($p < 0.001$). No significant association between FM and TBBMC or BMD was found in T2DM patients. Sclerostin was positively associated with TBBMC ($p < 0.001$) as well as with BMD at all measured regions. LM was negatively associated with 25OHD3 ($p = 0,024$) and osteocalcin ($p= 0,025$). In multivariate analyses LM significantly predicted TBBMC as well as TB and hip BMD.
Conclusion: In T2DM patients, LM significantly predicted BMD at the TB and hip BMD. Further research is needed to determine whether maintenance of LM in T2DM patients can prevent fractures. The role of sclerostin and osteocalcin in the pathogenesis of bone fragility in T2DM patients required further research. (This work was supported by the Czech Ministery of Health IGA MZ ČR NT 11335-6/2010.)
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"Atypical" Atypical Femur Fractures and Use of Bisphosphonates

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Background*:* Atypical femur fractures (AFFs) present a rare but serious condition associated with use of bisphosphonates. Underlying mechanisms and clinical risk factors remain unclear. According to the diagnostic criteria formulated by the ASBMR, a lateral localisation of an AFF is required.
Methods: We present a patient who developed bilateral leg pain while using an oral bisphosphonate and aromatase inhibitor in the course of adjuvant treatment for breast cancer. Initially she was diagnosed with bone metastases and received radiotherapy on the right femur. However, the bilateral periosteal reactions of the subtrochanteric femur are highly suggestive of AFFs. Our case meets all criteria for AFF except that she presented with lesions at the medial site of the femur. Therefore, they could be best described as "atypical" atypical femur fractures.
Conclusion: Since the pathogenesis of AFFs is not fully understood, we cannot rule out that AFFs also occur in the medial femur or in other weight-baring bones. Hence we propose that medial stress reactions belong to a spectrum of atypical fractures associated with use of antiresorptive drugs. The localisation may depend on yet unknown biomechanical factors. We propose that these periosteal reactions of the subtrochanteric femur are in fact AFFs with uncommon medial localisation and could hence be considered "atypical" AFFs. We recommend being alert of AFFs in patients with bone pain and medial subtrochanteric lesions. More epidemiological studies are needed to investigate the occurrence of both medial and lateral AFFs and to gain more insight into its frequency and pathogenesis.
Disclosure: The authors declared no competing interests.

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Bone Mass Loss with HIV-Infection to Sexuality

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Background: STDs are increasing and control of HIV infection is getting better. Our aim was to determine if the combined use of calcitonin and etidronate influences bone mass loss.
Methods: We studied for 12 months 21 women who were 42 to 57 years old and had a bone mineral density at the lumbar spine between 146 mg/cc and 75 mg/cc, 10 were assigned to 10 mg of etidronate. Eleven were treated with 10 mg of etidronate and 200 UI of intranasal calcitonin.
Results: Mean mineral bone density at the lumbar spine was between -1 and -3 DS below the mean value for premenopausal women. After a treatment of 12 months no statistical significance difference was found among both groups as for the bone mineral density at the lumbar spine.
Conclusions: It is necessary to carry out a wider and longer study, among VIH-patients, but it seems that etidronate contribute advantages to decrease bone mass loss, at least, at lumbar spine, without calcitonin. This results can be interesting for VIH-infected, who are on a lot of medication and with ancient, actual and future sexual issues in relation to osteoporosis.
Disclosure: The authors declared no competing interests.

OSTEOPOROSIS: TREATMENT

P339 (OP12)

P340

Abstract withdrawn

P341 (OP14)

P342 (OP15)

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Long-term Follow-up of Percutaneous Vertebroplasty in Osteoporotic Compression Fracture: Minimum of 7 Years Follow-up

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Background: Percutaneous vertebroplasty is effective surgical method for treating osteoporotic compression fracture. We assessed the radiographic and clinical outcome of patients who underwent percutaneous vertebroplasty (PVP) in osteoporotic compression fractures with a minimum of 7 years follow-up retrospectively.
Methods: Between January 2000 and August 2007, 253 patients were treated with PVP for osteoporotic compression fracture at our department; 81 patients died during follow-up and 101 patients (177 vertebras) were available for follow-up for over 7 years. We analysed the clinical and radiologic outcome including cement feature.
Results: The mean follow-up period was 7.9 years. Clinical outcome by mean visual analogue scale (VAS) score revealed a decreased 4.9 points perioperatively. A decreased score was maintained over 7 years in 46% of patients. A new adjacent vertebral fracture was documented by 55 vertebral bodies in 35 patients. During the follow-up period, 81 patients (44.5%) in 182 patients died. Anterior body height in the last follow-up was improved about 0.3 mm compared with the preprocedural value, but was not statistically significant. Also, the focal kyphotic angle was reduced from 12.3° at the preprocedural state to 11.7° ° at the postprocedural state but was not statistically significant ($p > 0.05$). Out of the 101 cases, the 89 cases for whom the cement was injected into the vertebral body were kept in a stable condition. Seven cases of radiolucent line with decreased bone density in the adjacent area of cement and 5 cases of cement cracks accompanied with vertebral collapse were observed.
Conclusions: PVP for osteoporotic compression fracture is an efficient procedure for pain relief by long term follow-up. The cement injected vertebrae showed stable radiologic progression without significant changes in vertebral height or kyphotic angle.
Disclosure: The authors declared no competing interests.

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Closing the Gap in Osteoporosis Management: Implementation and Outcome Analysis of Secondary Fracture Prevention Programs

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We present three analyses relating to Secondary Fracture Prevention programs (SFPP) that have been instituted to address the gap in osteoporosis management: (1) Systematic review and meta-analysis of 42 publications on SFPPs published between 1996 and 2011: Outcome measures extracted included bone mineral density (BMD) testing and osteoporosis treatment initiation rates. Studies were grouped into 4 models from Type A (assessment & treatment) through to type D (patient education only). Meta-analyses demonstrated increased BMD testing (p=0.06) and treatment initiation rates (p=0.03) with increasing intervention intensity. (2) A 2-year RCT of 102 patients initiated on oral bisphosphonate therapy at a SFPP, randomised to 6-monthly follow-up with the SFPP (Group A) or primary care physician follow-up (Group B). Compliance & persistence were measured using claims data and their predictors analysed. At 24-months, medication possession ratio (MPR) and persistence were high and similar in both groups. In the adjusted analysis, patients in group A were not more likely to be compliant or persistent than those in group B, indicating that initiation of therapy within an SFPP is associated with high long-term therapeutic adherence. (3) In a 7-year prospective study, we determined predictors of re-fracture amongst 234 subjects managed by the Concord SFPP. In multivariate analysis, co-morbidity (HR 2.04 if >3, 95%CI 1.10-3.79), corticosteroid use (HR 1.75, 1.12-2.73), total hip BMD (HR 1.36 per 0.1g/cm^2 decrease, 1.08-1.70) and a MPR of <50% (HR 3.36, 1.32-8.53) were significantly associated with re-fracture, indicating patients with these criteria are at high re-fracture risk, requiring intensive management. Our results demonstrate that (a) intensive SFPPs (Type A) are effective in raising treatment rates; (b) following treatment initiation by the programme, patients are likely to adhere to therapy outside the SFPP; and (c) therapeutic compliance remains the major determinant of re-fracture.
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CaSR-Activating Tripeptide has Novel Anabolic Actions on Human Osteoblasts

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Two lines of evidence suggest that the calcium-sensing receptor (CaSR) in osteoblasts is a therapeutic target for the treatment of osteoporosis and reduction in fractures. Knockout of the CaSR in osteoblasts drastically reduced bone mass in mice (Chang,Sci Signal, 2008). Strontium, which has been shown to reduce fractures in randomised controlled trials, appears to act, at least in part, through the CaSR (Brennan, Brit J Pharm, 2009; Rybchyn, JBC, 2011). Divalent cations are not the only agents that activate the CaSR, which also responds to L-amino acids and small peptides (Broadhead, JBC, 2011). The current study assessed the effects of a tripeptide, S-methylglutathione (SMG), on signalling, proliferation, differentiation, and cell survival after H_2O_2-induced oxidative stress in primary human osteoblasts (HOBs) and HOBs immortalised by transfection with SV40 large T antigen. Bone cells were cultured in DMEM with 10% fetal calf serum and adapted to serum free media (1mM Ca^{2+}) for 24 hours. Alkaline phosphatase activity increased in both bone cell types after 12 days treatment with 20μM SMG compared with vehicle ($p<0.001$). Treatment with 20μM SMG for 24 hours also prevented significant increases in apoptosis,

measured by caspase-3 activity, in both bone cell types, in response to 2.5 h exposure to 50 μM H_2O_2 as an inducer of oxidative stress ($p<0.05$). Assessed via western blot, SMG increased phosphorylation of Akt at S^{473}, mTOR at S^{2448}, β-catenin at S^{552} (part of the canonical Wnt pathway) as well as Erk1/2 at Thr^{202}/Y^{204} in HOBs. Knockdown of the CaSR by siRNA in HOBs, reduced SMG-stimulated phosphorylation of Akt ($p<0.05$), mTOR ($p<0.01$) and β-catenin ($p<0.01$). These results support the proposal that the new CaSR-acting modulator increases bone cell differentiation and protects against oxidative stress. The data indicate a role for this or a related analogue as a potential novel therapy for osteoporosis in humans.

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Vitamin K2 Treatment Reduces Undercarboxylated Osteocalcin by 68 % in the Bone Marrow

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Background: Clinical studies have suggested, that vitamin K2 prevents bone loss and protect against fractures. Vitamin K2 is suggested to affect bone through the bone matrix protein osteocalcin (OC). OC is produced by the osteoblast in an undercarboxylated form (ucOC) that is carboxylated with vitamin K2 as a cofactor. Carboxylated OC promotes mineralisation of bone. The aim of the study was to investigate the effect of vitamin K2 on bone metabolism. Bone is affected by many factors, which may not be reflected or measurable in peripheral blood. We therefore wanted to investigate if bone formation markers in the bone marrow are affected by K2.

Methods: In this randomised placebo-controlled double blinded clinical trial, 142 postmenopausal women (60-80 years old) with osteopenia were treated with vitamin K2 (375 μg MK-7) or placebo for 1 year. Both groups also received vitamin D3 (38 μg/day) and calcium (800 mg/day). Bone marrow aspirates were obtained from 56 women at baseline and after three months of treatment. Bone marrow serum was analysed for OC, ucOC and procollagen 1, N-terminal propeptide (P1NP).

Results: Baseline levels of ucOC was 5.0 μg/l ±4.4; OC was 18.8 μg/l ± 5.8; P1NP was 66.4 μg/l ± 25.3, without differences between groups. After three months, ucOC was reduced by 68 % in the vitamin K2 treated group, compared with the placebo group ($p<0.001$). OC and P1NP did not change.

Conclusion: Three months of treatment with vitamin K2 reduces ucOC in bone marrow, but does not change total OC or PINP. This suggests that the effect of vitamin K2 on bone is not mediated by stimulation of bone formation, but through changes in the bone matrix.

Further analyses of bone markers and gene expression are needed to understand the exact underlying mechanisms.

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Efficacy of Combined Treatment with Alendronate (ALN) and Eldecalcitol, a New Active Vitamin D Analogue, as Compared with that of Concomitant ALN, Vitamin D Plus Calcium Treatment in Japanese Patients with Primary Osteoporosis

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Background: We compared the clinical efficacy and safety of combined treatment with alendronate (ALN) plus eldecalcitol (ELD) with those of treatment with ALN supplemented with vitamin D and calcium.

Methods: Osteoporotic 219 patients were randomly assigned to a group prescribed ELD at 0.75 μg/day plus ALN at 35 mg/week (ALN + ELD group), or a group prescribed vitamin D3 at 400 IU/day, calcium at 610 mg/day, and ALN at 35 mg/week (ALN + VitD group) for 48 weeks. Primary endpoint was the inter-group differences in lumbar spine BMD (L-BMD) at patient's last visit. Secondary endpoints included the differences in BMD at other sites and the bone turnover markers (BTMs) levels.

Results: L-BMD, total hip BMD and femoral neck (FN-BMD) increased from baseline by 7.30, 2.41 and 2.70% in the ALN + ELD group, and by 6.52, 2.27 and 1.18% in the ALN + VitD group, respectively. Inter-group differences of the L-BMD and total hip BMD values were not significant. The increase of the FN-BMD was larger in the ALN + ELD group than the ALN + VitD group. Reductions of the BTMs were greater in the ALN + ELD group than the ALN + VitD group. The increase in L-BMD strongly correlated with the baseline values of the BTMs in the ALN + VitD group, whereas, the L-BMD increased regardless of baseline BTM in the ALN + ELD group. The safety profile did not differ between the two groups.

Conclusion: Combination treatment of ALN plus ELD was more effective in reducing the BTMs and increasing the FN-BMD than ALN treatment with vitamin D3 and calcium.

*e-ADVANCED Study = Eldecalcitol; Alendronate plus vitamin D Vs. AleNdronate Combined with ElDecalcitol Study.

Disclosure: The authors declared no competing interests. AS has received consultant fee and honoraria from Astellas, Chugai and Taisho-Toyama. The study was funded by Taisho Pharmaceutical Co. Ltd.

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Spontaneous Fusion after Vertebroplasty and Kyphoplasty in Osteoporotic Compression Fracture

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Background: We found a spontaneous fusion after percutaneous vertebroplasty (PVP) or kyphoplasty in osteoporotic compression fractures and analsze the radiologic & clinical characteristics.
Methods: Between January 2000 and January 2013, 555 patients were treated with PVP or kyphoplasty for osteoporotic compression fracture in our department. We classified the spontaneous fusion as two groups. One is absolute spontaneous fusion group with at least three cortical continuity to adjacent vertebrae, the other is partially fusion group which progressed fusion compared with previous radiologic finding. We reviewed the plain film and analysed the radiologic characteristics of those patients with duration of fusion, location and extent of fused segments. A clinical characteristic by visual analogue score (VAS) compared to our previous report was checked.
Results: Among them, 54 patients (9.7%) had an absolute spontaneous fusion and 43 patients (7.7%) had partially fused on plane image. In absolute fusion group, the average duration of fusion was 19 months ranged of 3 to 48 months. Forty-six cases (85%) of absolute fusion patients had occurred with proximal adjacent vertebrae and 7 cases (13%) had proximal with distal adjacent vertebrae. 41 cases (76%) of spontaneous fusion occurred within 1 segment and 13 cases within multiple segments. The most cases of absolute fusion group were occurred at thoracolumbar junction.(40 patients, 74%) Mean VAS score of absolute fusion group was 2.0 at final follow-up and were analysed relatively low score compared with mean VAS of our previous report (2.0, 2.8, respectively).
Conclusion: After percutaneous vertebroplasty or kyphoplasty in osteoporotic compression fracture, unpredictable spontaneous fusion could develop more than 10% rate, especially with proximal vertebra within 1 segment at thoracolumbar junction in radiologic aspect. Clinically, patients with spontaneous fusion had a tendency of more reduced pain than others.
Disclosure: The authors declared no competing interests.

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The Effect of Bisphosphonate Therapy on Serum Levels of Pyridoxal-5 Phosphate: Relevance to Atypical Femur Fractures

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Hypophosphatasia, a genetic disorder characterised biochemically by low activity of alkaline phosphatase (BAP) and high levels of phosphate esters including pyridoxal 5-phosphate (PLP), is associated with mineralisation defects and subtrochanteric femoral fractures. The latter suggest that bisphosphonates (BPs) should be avoided in patients with adult-onset hypophosphatasia as bisphosphonates might further inhibit alkaline phosphatase activity by binding zinc and magnesium. The aim of the study was to determine the effect of BPs on PLP levels. Ninety-two women with postmenopausal osteoporosis (> 5 years since menopause, < 85 years old) were randomized to treatment for two years with one of three amino-bisphosphonates, alendronate (70 mg weekly, n=33), ibandronate (150 mg monthly, n=28) or risedronate (35 mg weekly, n=31) as well as supplemental vitamin D and calcium. A control arm comprised 21 healthy women aged 35-40 who remained untreated for 2 years. We measured BAP (iSYS, IDS) and PLP (HPLC, normal<100) at baseline and 2 years and show the geometric means (and 95% confidence intervals) in Table 1.
There was a 43% decrease in BAP ($p<0.001$) in the osteoporotic women treated with bisphosphonate, but no change in the controls. There was no change in PLP in either group. A 78-year old woman treated with alendronate for an extra 3 years developed an atypical fracture of the left femoral shaft from among the 92 women and she had a normal initial PLP (75 nmol/L) and this was unchanged (67) after two years. We conclude that amino-bisphosphonate therapy in osteoporosis patients has no effect on the hydrolysis of phosphate esters by alkaline phosphatase.
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Table 1 [P349]

	BAP, ng/mL	BAP, ng/mL	PLP, nmol/L	PLP, nmol/L
	Baseline	2 years	Baseline	2 years
Osteoporosis	18.9 (17.4-20.4)	10.8 (10.1-11.6)	63 (56-70)	57 (51-65)
Controls	9.6 (8.1-11.2)	10.1 (9.2-11.2)	63 (48-83)	68 (52-89)

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Percentage of Women Achieving Non-Osteoporotic BMD T-Scores at the Spine and Hip during up to 8 Years of Denosumab (Dmab) Treatment

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Osteoporosis treatment guidelines currently do not define treatment targets or goals. While absence of BMD loss and fracture are generally considered treatment successes, lack of a negative outcome does not set a real goal for therapy. Potential goals might include reaching a BMD T-score somewhere above –2.5, representing an acceptable level of fracture risk. We report the percentage of women who achieved a range of possible target BMD T-scores at both the lumbar spine (LS) and total hip (TH) during up to 8 years of continuous DMAb treatment. Women received 3 years of DMAb (60 mg SC Q6M) during FREEDOM and up to 5 years of DMAb during the Extension for up to 8 years continuous treatment. The percentages of women achieving BMD T-scores >–2.5, >–2.0 and >–1.5 at both the LS and TH, and T-scores >–2.5 at either site over 8 years were determined. Among FREEDOM Extension participants (N=2343), mean (SD) LS and TH T-scores were –2.83 (0.67) and –1.85 (0.79), respectively, at FREEDOM baseline. The percentage of women with BMD T-scores >–2.5, >–2.0, and >–1.5 at both the LS and TH progressively increased over 8 years of DMAb treatment (Table 1).

From baseline through 8 years of DMAb treatment, the percentage of women with a BMD T-score >–2.5 increased from 19% to 86% at LS and from 75% to 94% at TH. In conclusion, DMAb enabled a substantial proportion of women with osteoporosis to achieve non-osteoporotic BMD T-scores. Furthermore, the BMD T-scores achieved at the hip during denosumab treatment are a robust predictor of the subsequent non-vertebral fracture risk, and suggest that achieving T-scores of -2.0 or higher are desirable to maximise treatment efficacy. These data contribute insightful information to discussions on the topic of treatment goals for osteoporosis.

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Table 1 [P350]: Percentage of Women Achieving Non-osteoporotic BMD T-scores at LS and TH.

	Baseline	Year 3	Year 4	Year 5	Year 6	Year 8
>-2.5	11%	57%	63%	69%	73%	82%
>-2.0	2%	23%	29%	36%	41%	53%
>-1.5	<1%	5%	8%	11%	15%	23%

P351

The Effect of Bisphosphonate Treatment on Bone Turnover and Bone Balance in Postmenopausal Women with Osteoporosis

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Postmenopausal osteoporosis is characterised by increased bone turnover, a negative balance (resorption>formation) and increased fracture risk. Bisphosphonate treatment reduces bone turnover and fracture risk but their effect on bone balance is yet to be fully investigated. We have compared the effects of bisphosphonates on turnover and balance in postmenopausal women with osteoporosis using a T-score bone marker method. 165 postmenopausal women (hip and spine BMD T-score ≤-2.5 or <-1 with a prior fracture) were recruited, mean age 67years. They were provided with calcium and vitamin D supplements and randomised to receive ibandronate (n=55, 150mg/month), alendronate (n=54, 70mg/week) or risedronate (n=56, 35mg/week). A fasting serum sample was collected at baseline and weeks 1,2,4,12,13 and 48 on treatment. The control group were 200 healthy premenopausal women receiving no treatments. PINP and CTX were measured using the iSYS-IDS analyser. Values were $\log_{10}$-transformed and normalised. The T-scores for PINP and CTX value were calculated for each postmenopausal woman using the mean and standard deviation values from the premenopausal group. By week 48 bisphosphonates reduced mean levels of turnover to -2.1SD units (95% CI:-2.322,-1.848) below the mean of the premenopausal women $p<0.001$. Mean levels of balance were positive (0.3SD units, 95% CI: 0.145, 0.559), $p<0.01$. Mean levels (95% CI) of balance and turnover for each bisphosphonate are shown in the table. By week 48 turnover decreased, $p<0.001$ and balance was more positive with alendronate only, $p<0.01$. In postmenopausal osteoporosis treatment with bisphosphonates improves bone balance by making it more positive and reduces bone turnover, relative to healthy premenopausal women. Bisphosphonates have differing effects on turnover and balance.

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Table 1 [P351]

Visit	Ibandronate	Alendronate	Risedronate
Balance baseline	0.205(0.005,0.405)	-0.060(0.262,0.141)	-0.004(-0.202,0.194)
Balance week48	0.173(-0.180,0.527)	0.597(0.259,0.935)**	0.248(-0.106,0.601
Turnover baseline	1.553(1.336,1.770)	1.342(1.123,1.562)	1.218(1.003,1.433)
Turnover Week48	-2.342(-2.740,-1.945)***	-2.441(-2.822,-2.061)***	-1.439(-1.836,-1.041)***

P352

Bone Loss from the Skull in Response to Teriparatide Therapy: Regional Differences in Response to Anabolic Osteoporosis Therapy

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Teriparatide is the active fragment (1-34) of human parathyroid hormone and it is one of the few anabolic agents licensed for the treatment of severe postmenopausal osteoporosis. It stimulates both bone formation and bone resorption and so has the potential to cause bone gain or bone loss. Large increases in spine BMD have been reported with this treatment, but its effects on total body BMD and the BMD of its anatomical sub-regions, for instance in the skull, have yet to be described. Women (n=18, age = 65.8 ± 5.1 years) with postmenopausal osteoporosis, defined as a BMD T-score of ≤-2.5 at the hip or lumbar spine by dual energy X-ray absorptiometry (DXA) were recruited. They were treated for one year with teriparatide by subcutaneous injection at the licensed dose (Forsteo 20 mcg daily, Lilly, Basingstoke, UK). We measured total body BMD by DXA at baseline and then at 12, 26 and 52 weeks. By 52 weeks, total body BMD had decreased by 1.3% (95% CI: -2.6 to -0.1%, p=0.05). When considering the anatomical sub-regions of the total body, two sites showed a statistically significant change in BMD. There was an increase in BMD at the lumbar spine (mean=9.5%, 95% CI: 6.0 to 12.9%, p<0.001) and a decrease in BMD in the skull (mean = 5.2%, 95% CI: -8.1 to -2.3%, p<0.01). We conclude that the BMD response to teriparatide differs by site with an increase at the spine and a decrease at the skull.

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P353

Individual Targeted Therapy of Vitamin D Deficiency in Postmenopausal Women

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Background: The aim was to assess the efficacy and safety of individual targeted vitamin D therapy in postmenopausal women with skeletal diseases (systemic osteoporosis and osteoarthritis).

Methods: Individual targeted therapy of vitamin D deficiency consists of two periods – saturation period and maintenance therapy period, during which patients take it constantly. Duration of saturation therapy is calculated by the formula: Saturation therapy duration (days) = (100 – blood 25(OH)D level (ng/mL)) X body weight (kg) /100. The therapy for saturation includes combined calcium (1000 mg of calcium and 800 IU of vitamin D) and 3000 IU of vitamin D per day. Maintenance therapy includes 2 000 IU of vitamin D per day. The study involved 70 postmenopausal women aged 46-87 years. All patients were divided into two groups: main - 50 women who took individual targeted vitamin D therapy (50 subjects, 65.1±8.8 years old, BMI 27.22±4.51 kg/m2) and control (20 subjects, 64.5±11.1 years old, BMI 26.68±4.95 kg/m2). The duration of the treatment consists of 3 months starting on the 1st Oct 2013.

Results: In 3 months after the start of the treatment there was a significant (p<0.001) increase in 25(OH)D levels in the treatment group: 35.60±8.21 nmol/L as compared with baseline levels of 25.20±9.76 nmol/l. Remarkably, the treatment was most effective in the oldest subgroup (>70 yrs.), as well as in subjects with the BMI 25-28,99 kg/m2. After the treatment, there were no changes in calcium levels.

Conclusions: The suggested individual targeted vitamin D therapy was proven to be effective in postmenopausal women. As the treatment turned out to be effective, relatively quick, and had a reasonable safety profile it may be beneficial for all vitamin D deficient postmenopausal women.

Disclosure: The authors declared no competing interests.

P354

Positive Association between BMD Gain by Risedronate Treatment and Early Suppression in Bone Turnover Markers (TRACP-5b, BAP): Sub-analysis of Japanese Phase III Trial of Risedronate 75mg

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Bone turnover markers (BTMs) are useful to monitor response and adherence to osteoporosis treatment. However, the relationship between risedronate-related suppression of BTMs and corresponding increases in bone mineral

density (BMD) is still unclear. This study further explored the nature of these relationships. Sub-analysis included 800 subjects of a Japanese phase III study in the patient with once monthly risedronate 75 mg treatment of 12 months who were measured of vertebral BMD, serum TRACP-5b (Tartrate-resistant acid Phosphatase 5b) and serum BAP (Bone alkaline phosphatase). The minimal significant change (MSC) was set at 12.4% for TRACP-5b and 23.1% for BAP. The subjects were divided into subgroups according to change in TRACP-5b or BAP at 1 and 3 months, i.e., into Group L (reduction more than MSC), Group N (change within MSC) and Group H (elevation more than MSC). Percent change in BMD at 12 months was compared among these subgroups. In TRACP-5b at 3 months, BMD gain was significantly greater in the Group L than the other groups (Group L [N = 661] 6.24 ± 4.11%, Group N [N = 99] 4.38 ± 4.50%, Group H [N = 33] 2.15 ± 4.21%, One-way ANOVA: $P < 0.0001$). In BAP at 3 month, BMD gain was significantly greater in the Gtoup L than other groups (Group L [N = 463] 6.52 ± 4.02%, Group N [N = 332] 4.97 ± 4.47%, Group H [N = 7] 2.26 ± 3.13%, One-way ANOVA: $P < 0.0001$). In summary, increases in BMD by risedronate treatment at 12 months were greater in the patient groups showing early reduction in TRACP-5b or BAP by more than MSC. This result suggests that serial measurements of BTMs at baseline and 3 months can be utilised in predicting future BMD gain by risedronate.

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P355

Efficacy and Safety of Clodronate in Renal Transplant Recipients with Suboptimal Renal Function and High Fracture Risk

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Background: Fracture risk is high after renal transplantation, but suboptimal renal function and associated high skeletal retention of potent nitrogen-containing bisphosphonates raise concern with their use. The less potent and less firmly bone bound second generation non-nitrogen containing bisphosphonate clodronate, shown to be effective in decreasing fracture risk in osteoporosis, represents an attractive and potentially safer alternative in these patients. The objective was to evaluate the efficacy and safety of one year oral clodronate in renal transplant recipients (Tx) with e-GFR ≥30 ml/min/1.73m2 and high fracture risk.

Methods: Tx patients with available DXA BMD and conventional spinal radiographs before and 1 year after starting clodronate were included. Demographic, laboratory and BMD data were collected. Spinal X-rays were evaluated for vertebral fractures (VF) using the Genant scoring system, and non-vertebral fractures (NVF) documented.

Results: Thirty-one Tx patients (18 female), mean age 44.8±9.2 yrs, time since Tx 4.3±5.3yrs, eGFR 56 ml/min/1.73m2 were studied. Mean serum values at start treatment were: creatinine 115±23 µmol/l, corrected calcium 2.71±0.18 mmol/l, PTH 23±18 pmol/l, alkaline phosphatase 146.6±99.6 IU/L, P1NP 99.5±65.2 ng/ml. Mean BMD T-scores were -1.68±1.66 at the lumbar spine (LS) and -1.86±1.38 at the femoral neck (FN). 6 patients had 9 VF and 5 patients had 7 NVF. After 12 months treatment, s.calcium decreased to 2.66 mmol/l (p=0.031), bone turnover normalized and renal function remained stable. BMD increased +2.0% at LS (p=0.066) and stabilised at FN. There were no new fractures. Treatment was well tolerated and there were no adverse effects.

Conclusion: Our data demonstrate that clodronate is effective and safe in renal Tx patients with eGFR>30 ml/min/1.73m^2, high bone turnover and high fracture risk. One year treatment significantly decreased serum calcium, normalised bone turnover, increased LS BMD, stabilised FN BMD, prevented further VF/NVF, and did not affect renal function. Whether these beneficial effects are maintained in the longer-term remains to be established.

Disclosure: The authors declared no competing interests.

P356

Denosumab (DMab) Restores Cortical Bone Loss at the 1/3 Radius Associated with Ageing and Reduces Wrist Fracture Risk: Analyses from the FREEDOM Extension Cross-over Group

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Cortical bone loss contributes importantly to increased fracture risk. DMAb increases BMD at sites of cortical bone, including the 1/3 radius, a site not responsive to most osteoporosis treatments. We evaluated changes over time in 1/3 radius BMD and wrist fracture incidence in women with postmenopausal osteoporosis during placebo and subsequent DMAb treatment. Wrist fractures were evaluated in 2207 women who enrolled in the FREEDOM Extension and received placebo during FREEDOM (3 years) and DMAb 60 mg Q6M during the Extension (6 years; cross-over

group). All women received daily calcium and vitamin D. A subset (n = 115) participated in a radius DXA substudy. Mean percentage changes in BMD over time were analysed using a repeated measures model and wrist fracture rates computed. At FREEDOM baseline, mean (SD) 1/3 radius T-score was –2.53 (1.18). While on placebo for 3 years, a significant loss of BMD at the 1/3 distal radius was observed (–1.2% at Year 3; $p < 0.05$ vs FREEDOM baseline), with a wrist fracture rate of 1.02 (95% CI=0.80–1.29) per 100 subject-years. Upon DMAb initiation, this bone loss was reversed, resulting in BMD gains at the 1/3 distal radius of 1.5% at Extension Year 2 ($p < 0.05$ vs Extension baseline). During the 3 years following DMAb initiation (Extension Years 1–3), the BMD deficit recovered to the original baseline levels and the wrist fracture rate stabilised. Over a further 2 years of DMAb treatment (Extension Years 4–5), BMD increased above the original FREEDOM baseline and significantly fewer wrist fractures occurred (rate ratio=0.57, 95% CI=0.34-0.95; p=0.03 vs FREEDOM placebo rate). This significant decline in the wrist fracture rate corresponded to the 1/3 radius cortical BMD improvement over baseline and continued until extension year 6 (rate ratio for Extension Years 4–6=0.61 vs FREEDOM placebo, 95% CI=0.39-0.94, p=0.025). These data provide additional evidence of a relevant clinical endpoint of reversing cortical bone loss in patients with osteoporosis.

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P357

Persistence with Denosumab in a Menopause and Osteoporosis Clinic

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Background: Poor persistence with oral bisphosphonates and other oral medications led to an increasing usage of intravenous regimens for the treatment of patients at risk of or with osteoporotic fractures. However, intravenous bisphosphonates may be accompanied by unpleasant side effects and inconvenient application. Therefore, subcutaneously administered Denosumab (DMAb) twice yearly was anticipated to possibly overcome those disadvantages and eventually improve persistence. The objective of this prospective observational study was to evaluate the persistence with DMAb in the real-world setting of a menopause and osteoporosis outpatient clinic in Austria.

Methods: 554 postmenopausal women with osteoporosis (defined by WHO criteria), 50 to 94 years of age, were studied to measure persistence with DMAb treatment between Sept. 2010 to Dec. 2014. Patients were monitored to evaluate tolerance and safety as well as efficacy by assessing side effects, adverse events and discontinuation of therapy as well as diverse parameters of bone metabolism in 6 months intervals.

Results: According to the cumulative numbers of patients lost to follow-up and to discontinuation of therapy, persistence was 92,2% at month 6, 88,1% at month 12, 85,4% at month 18, 83,0% at month 24, 81,4% at month 36 and 80,9% at month 48. The numbers of patients lost to follow-up and discontinuing therapy were highest at month 6 - 24 and 19 respectively out of 554 women - and decreased to 1 and 2 respectively out of 215 at month 36 and to zero out of 81 at month 48.

Discussion: The high rate of persistence with DMAb, in comparison to reported rates of other bone-specific regimens in the literature, may be attributed to a low frequency of side effects and adverse events with a concurrently high efficacy and a very comfortable way of administration.

Disclosure: The authors declared no competing interests.

P358

How the Uptake of Strontium and Fluoride in Relation to Osteoporosis Treatment affect the Composition, Structure and Mechanical Properties of Human Cortical Bone

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Strontium and fluoride treatment play a role as therapeutic options to antagonise fracture risk in osteoporosis. Incorporation of these elements in bone is accompanied by changes in remodelling, matrix composition and structure. However, a direct comparison of the effectiveness of strontium and fluoride treatment in human cortical bone with a focus on its mechanical properties remains to be established. Iliac crest bone biopsies obtained from autopsy cases without bone disease served as healthy controls. The study groups encompass iliac crest biopsies from treatment naïve osteoporosis cases, patients with diagnosed osteoporosis under sodium fluoride (NaF) or strontium ranelate (SrR) treatment. Using instrumental neutron activation analysis (INAA), a positive correlation between treatment period and uptake of strontium and fluoride was found. Quantitative backscattered electron imaging (qBEI) was carried out to investigate the degree of mineralisation and the cortical porosity. NaF- and SrR-treated patients showed a lower porosity of 10.9±7.7% and 10.3±6.2% in comparison to osteoporotic patients (18.1±13.2%) indicating an increased bone mass. The lowest porosity of 4.4±1.8% was observed in healthy controls. NaF and SrR treatment led to significantly higher bone mineralisation in comparison with treatment naïve osteoporotic bone (+9.2% and +9.5% in mean calcium content), while the highest mineralisation pattern was found in iliac crest cortices of healthy controls. Mechanical properties were assessed via reference point indentation (RPI) as a surrogate measure of bone toughness. RPI showed that incorporation of strontium and fluoride in comparison with non-treated

osteoporotic individuals led to significantly decreased indentation distance indices as a measurement of bone's resistance to deformation and micro-fractures. Healthy controls showed the highest resistance. In conclusion, we found that osteoporosis treatment with both NaF and SrR had positive effects on mechanical characteristics beyond a gain in bone mass but did not resemble the properties of healthy cortical bone in the iliac crest.
Disclosure: The authors declared no competing interests.

P359

Factors Related to the Response Rate of Bone Mineral Density (BMD) to Osteoanabolic Therapy (Teriparatide/PTH) in Patients with Severe Osteoporosis

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Background: Factors related to the magnitude of BMD response to osteoanabolic treatment remain unclear. Thus, this study evaluated the long-term BMD response rate to osteoanabolic treatment (teriparatide/PTH) in patients with severe osteoporosis and the factors related to "inadequate response" (IR).
Methods: Fifty-seven patients (52F:5M) aged 67.5±10.7 years receiving teriparatide (50) or PTH1-84 (7) during 18 or 24 months were included (83% had vertebral fractures [median 4], 72% non-vertebral fractures, 97% previously received antiosteoporotic treatment [mean duration 6.2±4.9years], 23% were on glucocorticoid treatment). Bone turnover markers (BTM) (formation: P1NP, bone ALP; resorption: sCTx, NTx) and 25-OHD levels were assessed before and at 3, 6, 12 and 18/24 months of treatment. Lumbar and femoral BMD and spinal X-ray were assessed at baseline, and at 12 and 18/24 months. Previous and incidental fractures, previous antiosteoporotic treatment, risk factors and cause of osteoporosis were recorded in all patients. IR was defined by a lumbar BMD increase <3% at 18/24 months. BTM were evaluated in fold-number over/under normal values and as a normalised bone formation/resorption index.
Results: 32% of patients showed IR, presenting higher baseline lumbar BMD values (0.824±0.156 vs. 0.733±0.140g/cm^2, p=0.04) and longer previous antiosteoporotic treatment (7.7±4.2 vs. 5.5±5.2years, p=0.043). No significant differences were observed in age, baseline BTM or bone formation/resorption index, and 25-OHD levels between patients with or without IR. IR patients presented worse BMD evolution in lumbar spine and femur at 18/24 months (Lumbar BMD: -2.8±4.2% vs.11.5±8.1%, p<0.001; total femur -0.6±2.2% vs.3.9±7.9%, p<0.001). No significant differences were observed in BTM changes throughout the study or in the evolution of their normalised bone formation/resorption index. 25-OHD levels and the incidence of new fractures were similar in both groups throughout the study.
Conclusion: In patients with severe osteoporosis, baseline BMD values and duration of antiosteoporotic therapy seem to influence the response to osteoanabolic agents.
Disclosure: The authors declared no competing interests.

P360

Compliance to Oral Bisphosphonate Therapy and Fracture Risk: Influence of Exposure Misclassification in Pharmacy Claims Data

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Background: Pharmacy claims data are commonly used to estimate drug exposure. We previously identified misclassification in days supply values that underestimated medication compliance, particularly in long-term care (LTC). In this study we examined the impact of oral bisphosphonate exposure misclassification in days supply values on estimates of drug effectiveness in reducing hip fractures.
Methods: We used Ontario administrative claims data to identify new users of oral bisphosphonates aged 66 or more years between 2001 and 2011. Medication compliance was quantified by the proportion of days covered (PDC) and categorised into groups according to a 365-day ascertainment period. PDC was calculated using observed and cleaned days supply values. Hip fracture rates within 365 days after the ascertainment period were calculated using Cox proportional hazard models, adjusted for behavioural and fracture risk factors. Low compliance (PDC<20%) was the referent. Analyses were completed overall and separately for patients in community and LTC settings.
Results: The rate of hip fracture was higher in LTC (2.4/100 patient-years) than in the community (1.0/100 patient-years). Overall, cleaning days supply values increased the estimated benefit of high compliance (PDC≥80%) on fracture prevention ($HR_{observed}$=0.74, 95% CI=0.66-0.83; $HR_{cleaned}$=0.65, 95% CI=0.57-0.74). Risk estimates were similar among community-dwelling patients ($HR_{observed}$=0.68, 95% CI=0.60–0.77; $HR_{cleaned}$=0.65, 95% CI=0.56–0.75), yet differed substantially in LTC before data cleaning ($HR_{observed}$=0.96, 95% CI=0.73–1.26; $HR_{cleaned}$=0.64, 95% CI=0.46–0.91).
Conclusion: Misclassified days supply values can bias estimates of drug effectiveness. Larger effects on risk estimates were noted in LTC, where fracture risk is highest. Overall, little change was noted in the community setting, where most studies are completed. Thus, results caution researchers to examine days supply accuracy in pharmacy claims data when estimating the relationship between drug exposure and health outcomes, particularly when including LTC settings.
Disclosure: The authors declared no competing interests.

P361

Uptake and Characteristics of Zoledronic Acid and Denosumab Patients and Physicians in Ontario, Canada: Impact of Drug Formulary Access

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Background: We sought to describe the use of zoledronic acid and denosumab by physicians and patients over time, and examine the impact of a 2012 provincial formulary modification that removed prescribing restrictions for zoledronic acid.
Methods: We identified users of zoledronic acid and denosumab using Ontario pharmacy claims data. The number of new patients and physicians were plotted and examined over time. Interrupted time-series analysis examined the impact of a formulary modification to zoledronic acid. Descriptive characteristics of patients and prescribers were summarised pre- and post-formulary change for zoledronic acid and overall for denosumab.
Results: We identified 1,463 zoledronic acid users treated by 627 physicians and 16,736 denosumab users treated by 2,904 physicians. In the first two months on the market, we identified a rapid uptake of denosumab (>450 new physicians and >1,200 new patients) in contrast to zoledronic acid (<10 new physicians and <10 new patients). Zoledronic acid use increased significantly in the two months post-formulary change with >80 new physicians and >200 new patients. Prior to the formulary change, more zoledronic acid users had a history of osteoporosis therapy (41% vs. 26%) or bone density testing (30% vs. 10%). Compared with zoledronic users initiating after the formulary change, more denosumab users had prior osteoporosis therapy (55% vs. 26%), yet fewer had a gastrointestinal diagnosis (6% vs. 11%).
Conclusion: We identified a rapid uptake of denosumab in only 15 months of observation. A provincial formulary modification removing administrative burden on physicians prescribing zoledronic acid resulted in an increase in utilisation and impacted patient characteristics.
Disclosure: The authors declared no competing interests.

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Fracture Incidence and Changes in Quality of Life and Back Pain in Patients with Osteoporosis Treated with Teriparatide: 24-month Results from the Extended Forsteo® Observational Study (ExFOS)

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Background: The aim was to describe the fracture outcomes, health-related quality of life (HR-QoL) and back pain (BP) of patients with osteoporosis treated with teriparatide for up to 24 months in the ExFOS study.
Methods: Prospective observational study in patients with osteoporosis from eight European countries. Treatment duration was up to 24 months. Low-trauma and any type of clinical vertebral and non-vertebral fractures were collected at each follow-up visit. HR-QoL was measured using EQ-5D. BP was measured using a 100 mm visual analog scale (VAS) and a BP questionnaire.
Results: 1,607 patients were enrolled, and 1,454 received at least one dose of teriparatide. Of these, 1,318 (90.6%) were female, and 210 (14.4%) were taking glucocorticoids. Mean age was 70.2 years. At baseline, 65.5% of patients had 2 or more pre-existing fractures.11.3% of the study participants were osteoporosis-treatment naïve. Mean treatment duration with teriparatide was 21 months. 67 patients (4.6%) sustained a total of 77 incident low-trauma fractures (23% vertebral, 77% non-vertebral). A 53% decrease in the odds of low-trauma clinical fractures in the last 18-24-month period compared with the first 6-month period was observed ($p<0.05$). The mean total EQ-5D Health state increased from baseline to 24 months ($p<0.001$). A reduction of BP was observed after 3 months (-10.2 mm) and sustained up to 24 months (-23.1 mm) ($p<0.001$). At baseline, 90.8% of patients experienced BP in the month preceding study entry. This frequency was reduced to 79.7% and 70.2% after 12 and 24 months respectively. Moderate/severe BP was reduced from 81.1% of the patients at baseline to 43.1% at 24 months.
Conclusion: Treatment with teriparatide in the ExFOS cohort resulted in a reduction of fractures and back pain and improvement of health-related quality of life over time. These results should be interpreted in the context of a non-controlled observational study.
Disclosure: AB is a full time employee of Eli Lilly Sweden. This work was funded by Eli Lilly and Company Europe.

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Medical Care of Patients at Risk of Glucocorticoid-Induced Osteoporosis: the GLUCOST Study

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Background: Osteoporosis prevention and treatment needs to be considered in all patients started on chronic oral glucocorticoids (≥3 months of treatment). The objective was to assess prevention and treatment of glucocorticoid-induced osteoporosis (GIOP) in Russian patients.
Methods: Cross-sectional study was conducted in 34 centers of Russia. 1129 patients (mean age 53,3±13,9, 235 male and 894 female) currently taking long-term oral glucocorticoid therapy due to different chronic inflammatory diseases completed a special questionnaire (including fractures, major risk factors for osteoporosis, knowledge of osteoporosis, BMD testing, prescriptions of specific osteoporosis drugs, calcium and vitamin D supplementation and their actual intake). The median duration of OGC therapy was 5 years, median daily prednisolone dose - 10 mg. The adherence to

published national published guidelines for GIOP (updated in 2009) has been evaluated.
Results: 61.8% participants knew about GIOP. Only 48.1% patients underwent BMD testing. Most patients (78.1%) remembered about the importance of obtaining an adequate amount of calcium and vitamin D, but only 43.4% received calcium and vitamin D supplements on a regular basis. 25,4% participants reported osteoporotic fractures. GIOP treatment was assigned to 50.8% of patients at high risk of fractures, but in reality, 40.2% of the patients received it. Diagnosis and treatment of osteoporosis were performed more rarely in men than in women. Participants who knew about osteoporosis were 2.7 times (odds ratio) more likely to take calcium supplements and vitamin D (95% confidence interval [CI] 2.1-3.5, p = 0,001) and 3.5 times more likely to comply with treatment (95% CI 2.3-5.3, p = 0.001). BMD testing increased the likelihood of anti-osteoporotic drug prescriptions by physicians (8.2[(95% CI 6.2-11.0, p<0,001]) and the use of drugs by patients (6,5 [95 % CI 4,8-8,8, p<0,001]).
Conclusion: The challenge of the physicians and patients education about the risks of GIOP is not solved.
Disclosure: The authors declared no competing interests.

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Musculoskeletal Effects of Chiropractic Manipulation in an Osteoporotic Animal Model

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Chiropractic is focused on the diagnosis, treatment and prevention of musculoskeletal disorders. Evidences suggest that chiropractic manipulation (CM) might exert positive effects in osteoporotic patients. Muscle tension changes during CM could account in part for these beneficial bony effects. The objective of this study was to evaluate the effects of CM on bone structure and skeletal muscle in ovariectomised rats. Sham-operated (Sh; n =10) and ovariectomised (OVX; n =15) Sprague-Dawley rats (6 months-old) were submitted (M group) or not (NM group) to CM using the activator adjusting instrument model 5 (ACT5) as follows: Force 1 setting was applied onto the tibial tubercle of the right hind limb of M-rats (true CM; tM), at an angle of approximately 90º, from medial to lateral ; the corresponding left hind limb received a sham CM consisting of firing the ACT5 in the air and then slightly touching the tibial tubercle. These procedures were repeated 3 times a week for 6 weeks. BMD and BMC in long bones and L3-L4 vertebrae were determined in the living subjects. After euthanasia, femora and tibiae were removed for μCT analysis; *Soleus*, quadriceps and tibial muscles were submitted to Western analysis of mechano growth factor (MGF) protein. No differences in all parameter studied were observed between NM-OVX and fM-OVX or between NM-Sh and fM-Sh groups. The decrease of BMD and BMC as well as trabecular bone impairment in the long bones of NM-OVX rats, compared to NM-Sh group, was partially reversed in tM-OVX rats. This was accompanied by higher MGF protein expression in quadriceps and anterior tibial muscle of the latter group compared with NM-OVX. Bone mass values of L3-L4 vertebrae were similar in all OVX-groups, independent of CM. These results support the hypothesis that CM may improve osteoporotic bone through a mechanism involving skeletal muscle.
Disclosure: The authors declared no competing interests. This work was supported by Real Centro Universitario Escorial-María Cristina (N/A).

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Association of Gastrointestinal Events and Treatment Satisfaction Among Osteoporotic Women: Results of the Medication Use Patterns, Treatment Satisfaction and Inadequate Control of Osteoporosis Study (MUSIC-OS)

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Background: MUSIC-OS is a prospective, observational study which investigates the burden of gastrointestinal (GI) events and association with treatment (Tx) satisfaction, adherence, healthcare resource use, and quality of life among osteoporotic (OP) women in Canada, France, Italy, the Netherlands, Sweden and the UK. The objective of our study was to examine the association of GI events on OP Tx satisfaction among osteoporotic women.
Methods: Osteoporotic women >=50 years treated with OP medication were enrolled in the study during routine physician visits. Women diagnosed with Parkinson's disease, neuromuscular disease, Paget's disease, malignant neoplasm or treated with any injectable OP medication at enrolment were excluded. Women treated for OP were asked to complete the OP Patient Satisfaction Questionnaire (OPSAT-Q) regarding their OP Tx satisfaction at baseline (BL) and 6 and 12 months post-BL. Both new users (same OP Tx for <90 days) and experienced users (same OP Tx for >90 days) were included. Multivariate regression analysis estimated the association between GI events and OP Tx satisfaction after controlling for demographics and clinical characteristics.
Results: 2,943 women with mean age of 69.5 years were enrolled. Women who reported a GI event at six months after BL, but did not report any GI event at BL, had significantly lower OPSAT-Q scores compared with women who had not reported a GI event at 6 months after BL (LS Means difference: -3.97, p=0.0004, 95% CI: -6.16, -1.78). New users with GI events at 6 months after BL had lower OPSAT-Q scores

compared to those without GI events (LS means difference: -6.11, p=0.0062, 95% CI:-10.49,-1.74). Experienced users with GI events at 6 months (213 of 700, 30.4%) also had lower Tx satisfaction than those without GI events (LS means difference: -3.61, p=0.0051, 95% CI:-6.14, -1.08).
Conclusion: Results demonstrate that experience of new GI events may lower OP Tx satisfaction among osteoporotic women.
Disclosure: Employees of Merck & Co., Inc. have contributed to the design and implementation of this study. This study is fully funded by Merck & Co., Inc.

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The Impact of Gastrointestinal Events on Osteoporosis Related Health Care Resource Use Among Osteoporotic Women: Results of The Medication Use Patterns, Treatment Satisfaction and Inadequate Control of Osteoporosis Study (MUSIC-OS)
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Background: MUSIC-OS investigates the burden of gastrointestinal (GI) events in osteoporotic women in terms of the impact on healthcare resource use (HCRU), treatment (Tx) satisfaction, adherence, and quality of life. Our objective was to examine the impact of GI events on osteoporosis (OP) related HCRU in osteoporotic women at 3 and 6 months after study entry (baseline, BL).
Methods: This prospective, observational study enrolled 2,943 participants in Canada, France, Italy, the Netherlands, Sweden and the United Kingdom. Women treated for OP were asked to report their OP related resource use at BL, and 3, 6 and 12 months post-BL.HCRU was measured by number of physician visits. Multivariate regression analysis was conducted to understand the impact of GI events on subsequent OP HCRU.
Results: The mean age of women in the analysis was 69.5 years. 938 of 2,943 (31.9%) women did not report GI events at BL. Of these, 211 (22.5%) reported a GI event by 3 months post-BL. These women had significantly higher use of OP related health care resources for their OP, compared with women who had not reported a GI event by 3 months post-BL (OR 1.5, p=0.0529, 95% CI: 1.00, 2.25). These results persist at the 6 month time point. Women who did not report a GI event at BL but had reported one by 6 months post-BL (306, 32.6%) had higher odds of OP HCRU (OR 2.33, p=0.0001, 95% CI: 1.53, 3.55), and higher odds of OP primary care HCRU (OR 1.89, p=0.028, 95% CI: 1.07, 3.34), compared with those who never had a GI event.
Conclusion: 32.6% of women without prior GI events experienced a GI event by 6 months post-baseline, resulting in higher odds of OP related HCRU compared with those who didn't have a GI event.
Disclosure: Employees of Merck &Co., Inc. helped design and provided authorship to this study. This study was fully funded by Merck & Co. Inc.

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Efficacy of Fortified Bread in Vitamin D Deficient Postmenopausal Women
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Background: Vitamin D deficiency (VDD) is a very widespread syndrome associated with the development of multiple diseases; at the same time it is oftentimes neglected by the society and even healthcare professionals, yet is relatively easy to correct by administration of fortified products.
Methods: Two separate studies involving postmenopausal women have been conducted: one pilot study (22 subjects aged 50-80 years old) and one comparison study (30 subjects 45-80 years old). We established the total 25(OH)D level in serum, as well as parathyroid hormone, calcium, phosphor, alkaline phosphatase, and lipids levels according to the commonly utilised methods. All subjects received 277g of bread (daily amount) with or without fortification by 25mcg of vitamin D.
Results: Vitamin D daily intake in control group was 0.61 [0.31; 0.83] mcg (norm – 2.5 mcg); thus optimal vitamin D levels in blood serum were registered in 13.3% of women only. Intake of fortified bread has facilitated a significant increase in 25(OH)D levels (p<0,001), as well as ionised calcium increase (p=0,03) in blood serum; this change has reached 41.2% in patients with VDD and 15.6% in patients with vitamin D insufficiency.
Conclusion: The product has adequate taste, triggers significant 25(OH)D level increase in serum and is not associated with adverse events development.
Disclosure: The authors declared no competing interests.

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Comparison of Treatment Responder Rates for Three Oral Bisphosphonates: the TRIO Study
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Bone mineral density (BMD) is used to assess the efficacy of bisphosphonate treatment in the individual patient, however it is unclear whether the response rate is similar for all oral bisphosphonates. The TRIO study is a randomised, open-label, parallel, controlled trial of three bisphosphonates, orally administered at their licensed dose for two years. We recruited postmenopausal women (n=172, ages 53-84 years) with a BMD T-score, by DXA, of ≤-2.5 at the spine and/or total hip, or of ≤-1.0 plus a previous low trauma fracture. Women were randomised to receive one of three bisphosphonates,

Table 1 [P368]: The proportion and percentage of responders by treatment duration and bisphosphonate type.

	Week 12		Week 48		Week 96	
	Proportion of responders (n/group n)	Responders (%)	Proportion of responders (n/group n)	Responders (%)	Proportion of responders (n/group n)	Responders (%)
Lumbar spine						
Ibandronate	10/49	20.4	24/45 [a]	53.3	20/29 [b]	69.0
Alendronate	11/35	20.0	25/50 [a]	50.0	29/34 [b]	85.3
Risedronate	7/47	14.9	12/46 [a]	26.1	13/30 [b]	43.3
All	28/152	18.4	61/141	43.3	62/93	66.7
Total hip						
Ibandronate	6/48	12.5	10/44	22.7	9/28 [c]	32.1
Alendronate	10/55	18.2	12/50	24.0	18/34 [c]	52.9
Risedronate	3/48	6.3	4/46	8.7	6/30 [c]	20.0
All	19/152	12.5	26/141	18.4	33/92	35.9

Number of responders is significantly different between bisphosphonates by chi-squared testing; [a](p=0.02), [b](p=0.002) and [c](p=0.02). Number of responders is significantly different between bisphosphonates by chi-squared testing; [a](p=0.02), [b](p=0.002) and [c](p=0.02).

either alendronate 70 mg once weekly, risedronate 35 mg once weekly, or ibandronate 150 mg once monthly, together with calcium (1200 mg) and vitamin D (800 IU) daily supplements. Lumbar spine and total hip BMD were measured at baseline and weeks 12 (in duplicate), 48 and 96. We calculated changes in lumbar spine and total hip BMD between baseline and weeks 12, 48 and 96 for each individual. The duplicate week 12 results were used to calculate the least significant change (LSC) for lumbar spine (4.4%) and total hip BMD (4.2%). Women could be classified as treatment responders if their individual BMD increase was greater than the site-specific LSC. Differences in the number of responders by weeks 12, 48 and 96 were examined using chi-squared tests (Table 1). Even though the LSCs were similar, lumbar spine BMD was able to identify more treatment responders than total hip BMD. We found that the number of treatment responders was dependent on the bisphosphonate type. By week 96, more women had responded to alendronate than ibandronate and risedronate.

Disclosure: The authors declared no competing interests. This work was funded by Warner Chilcott.

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The Epigenetically Active Small Chemical N-Methyl Pyrrolidone (NMP) Prevents Ovariectomy-Induced Osteoporosis in Rats

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Osteoporosis is a chronic skeletal disorder, prevalent in postmenopausal women influenced by hormonal factors causing a huge economic burden on our ageing society. Currently, there are several treatments for osteoporosis however; they all display some sort of limitation and/or side effects making the need for new treatments imperative. We have previously demonstrated that NMP is a bioactive drug which enhances bone regeneration *in vivo* and acts as an enhancer of bone morphogenetic protein (BMP) *in vitro.* NMP also inhibits osteoclast differentiation and attenuates bone resorption. In the present study, we tested NMP as a bromodomain inhibitor and for osteoporosis prevention on ovariectomised (OVX) induced rats while treated systemically with NMP. Female Sprague-Dawley rats were ovariectomised and weekly NMP treatment was administrated 1 week after surgery for 15 weeks. Bone parameters and related serum biomarkers were analysed. Fifteen weeks of NMP treatment slowed down body weight gain and prevented bone loss of the treated group compared with the control. In the same time both deterioration of overall bone loss and trabecular microstructure were preserved. Moreover, mineral apposition rate and bone biomarkers of bone turnover in the treatment group were at similar levels with those of the Sham group. Due to the function of NMP as a low affinity bromodomain inhibitor and its mechanism of action involving osteoblasts/osteoclasts balance and inhibitory effect on inflammatory cytokines, NMP is a promising therapeutic compound for the prevention of osteoporosis.

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GOS/FOS® as a Tool to Improve Bone Health in a Model of Osteopenia

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Same nondigestible oligosaccharides (NDOs) stimulate Ca and phosphorus (P) absorption and improve bone mass. The effect of NDOs appears to be more important when the diet does not meet Ca nutritional recommendations. Oestrogen deficiency induces a loss of bone mass which is greater if the intake of Ca is insufficient. The objective was to evaluate the effect a mixture of galactooligosaccharides (GOS) and fructooligosaccharides (FOS) (GOS/FOS®) (9:1) added to a low or a normal Ca diet on Ca and P absorption, bone retention, mineralisation and structure in osteopenic-oestrogen deficient rats. Female adult Wistar rats were OVX and remained untreated for 45 days to become osteopenic. Then, they were randomly assigned to receive for an additional 45-day period (T90) one of the following treatment: C5: AIN93-M containing 0.5% Ca; P5: C5 +2.5% GOS/FOS®, C3: AIN93-M containing 0.3% Ca; P3: C3 +2.5% GOS/FOS®. Body weight (BW) and intestinal

lactobacilli count (UFC) were evaluated weekly. Ca and P absorptions were assessed at baseline and T90. Caecum pH, femoral Ca and P content, total skeleton BMC and BMD (Lunar DXA), bone volume (BV/TV), bone-breaking strength, elastic modulus and stiffness were determined at T90. Results: No differences in BW were observed. Caecum pH decreased while UFC ($p<0.0001$); Ca and P absorption ($p<0.05$); femur Ca and P content ($p<0.01$); BMC ($p<0.05$); BV/TV; bone-breaking strength; elastic modulus and stiffness ($p<0.05$) increased in P5 and P3 vs. C5 and C3, respectively. In conclusion, the used GOS/FOS® mixture increased Ca bioavailability that improved bone health during oestrogen withdrawal.
Disclosure: Grant of PIP004 and ® N.V.Nutricia.

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Guidelines on the Management and Treatment of Glucocorticoid (GC)-induced Osteoporosis (GIO): from the Japanese Society for Bone and Mineral Research (JSBMR)

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Glucocorticoid (GC)-induced osteoporosis (GIO) is the most common secondary osteoporosis and fractures occur in 30 to 50 % of patients receiving GC therapy. Although it is important to prevent bone loss and fragility fracture by the early intervention, adherence to guidelines on the management of GIO is about 20% in Japan. We, therefore, revised the guideline based on accumulated references and collected data from 5 Japanese cohorts of GIO. By the analysis of 903 patients from 3 cohorts, age, GC dose, lumbar BMD and prior fragility fracture were identified as factors that predicted future fractures. When the hazard ratio for age was calculated versus <50 years, the fracture risk was 1.446 times higher at age 50≤ <65 and 2.108 times higher at age ≥65. Similarly, hazard ratio of fracture risk was as follows; GC dose 5≤ <7.5: 1.149, ≥7.5: 2.166 vs. < 5 mg/day; %YAM of lumber BMD 70≤ <80: 1.373, <70: 1.863 vs. ≥80; with prior fragility fracture: 3.485 vs. w/o it; with bisphosphonate 0.481 vs. w/o it. The parameter estimates for each risk factor were converted to tentative scores by the formula. The optimal cut-off score for setting the intervention threshold was validated by the data of 144 patients from 2 cohorts on primary prevention and determined by the careful discussion in the committee. It covers patients ≥18 years who use or are planning to use GC for ≥3 months and the intervention is based on achievement of a total score of ≥3 from each score in 4 domains: prior fragility yes: 7, age 50≤ <65: 2 and ≥65: 4, GC 5≤ <7.5: 1 and ≥7.5: 4, %YAM of lumber BMD 70≤ <80: 2 and <70: 4. Thus, it will aid the physician in easy and adequate decision-making for initiation of intervention to prevent fragility fracture due to GIO.
Disclosure: Y. Tanaka, has received consulting fees, speaking fees, and/or honoraria from Abbvie, Chugai, Astellas, Takeda, Santen, Mitsubishi-Tanabe, Pfizer, Janssen, Eisai, Daiichi-Sankyo, UCB, GlaxoSmithKline, Bristol-Myers and has received research grants from Mitsubishi-Tanabe, Chugai, MSD, Astellas, Novartis.

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Comparison between Alendronate Oral Jelly and Alendronate Weekly Tablet in the Treatment of Osteoporosis in Japan

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At this point, alendronate oral jelly is available only in Japan. We conducted the comparative trial of alendronate oral jelly (ALN-J) versus alendronate weekly tablet (ALN-T) to clarify the effect of alendronate oral jelly in the treatment of osteoporosis in Japanese clinical settings. The patients were assigned to ALN-J or ALN-T group in accordance with their request in each institution and observed for 6 months. The endpoints were bone turnover marker, bone mineral density (BMD), safety and others. As a result, 94 patients were enrolled in this trial from ten institutions, and 65 patients (female, 62; male, 3) assigned to the ALN-J group and 29 patients (female, 27; male, 2) to the ALN-T group. Mean [SD] of age was 77.5 [7.6] in the ALN-J group and 76.0 [6.6] in ALN-T group, and proportion of history of fracture was over 70 % in each group. TRACP-5 decreased by 38.8 % in the ALN-J group and 36.8 % in the ALN-T group at 3 months and 44.0 % in both groups at 6 months. P1NP decreased by 44.2 % in the ALN-J group and 45.9 % in the ALN-T group at 3 months, and 51.6 % and 57.8 % at 6 months, respectively. Lumbar spine BMD increased by 3.6 % in the ALN-J group and 4.2 % in the ALN-T group at 6 months, hip BMD 1.3 % and 0.8 %, radius BMD 0.9 % and -0.1 %, respectively. Alendronate oral jelly has equal effectiveness to alendronate weekly tablet.
Disclosure: The authors declared no competing interests.

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Implementation of Care for Osteoporosis Patients in the Netherlands: the Dutch Network of Fracture Liaison Services

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The fracture liaison service (FLS) is advocated as the most appropriate approach for secondary fracture prevention, but its implementation is variable between hospitals and countries. The International Osteoporosis Foundation (IOF) has proposed standards to evaluate the implementation of FLS. We did send an anonymous questionnaire based on the IOF FLS standards to 90 non-university hospitals in the Netherlands, of which 24 (27%) fully responded and results were assessed. In the 24 hospitals, 24,468 consecutive patients of 50 years and older with a recent fracture, representing around 25% of all fractures in the year 2012 in the Netherlands, were identified. After excluding patients with skull or toe fractures and upper age limits variable for each FLS, 11.983 (49%, range: 19%-95%) were able and willing to be

examined. High implementation of IOF standards (>90%) was achieved for fracture patient identification, invitation for FLS, timing of assessment, identification of vertebral fractures, application of national guidelines, evaluation of secondary osteoporosis, drug initiation when indicated, communication with the general practitioner, and application of a follow up strategy. Noteworthy, some standards are highly variable (evaluation of secondary osteoporosis), difficult to apply (for vertebral fractures and fall prevention) or not attainable (for achieving a patient response rate above 90%). We conclude that all patients attending the FLS were evaluated, treated and followed with high implementation of IOF standards. The major shortcoming of the study outcome is the low response rate of invited patients to attend the FLS. Reasons for this low response rate and how to increase it, need further study. Some standards were difficult to apply and analyse, and therefore could need adaptation.
Disclosure: The authors declared no competing interests.

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Factors Influence the Adherence to Recommended Osteoporosis Prevention Measures in Women in the Russian Federation
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Background: Despite high prevalence and severity of complications, osteoporosis (OP) in Russia is still not recognised as a socially significant disease and there are no common standards for prevention of OP and associated fractures. The aim was to investigate the adherence of healthy females to prevention measures of OP and the affecting it factors to develop the principles governing the preventive strategy of OP.
Methods: The study is performed in Moscow Region (MR) in a form of a questionnaire survey in the population of adult healthy women aged >20 years old. The data are obtained using "Questionnaires on osteoporosis prevention" and "The test of knowledge in the field of osteoporosis" developed in Clinical Research Institution of Moscow Region named after M.F. Vladimirsky. Study comprised 1712 females living in MR aged of 20-87 years old, median 55.0 years [45.0; 44.0]. Taking into account the quality of the filling of questionnaires, data provided by 1631 women were included in the statistical analysis.
Results: It is established that 31% of female inhabitants of MR are engaged in OP prevention, using for this purpose mainly calcium-containing drugs (64.3%) and increased consumption of calcium in food (59.8%). 93% of these women get preventive therapy at their own expense, spending on them on the average 200 rub a month, and preventive medication has appeared more saving than non-pharmacological preventive measures. Socially active working women aged of 50-69 years old are motivated on prevention of OP better than others. Level of the OP awareness and undertaking DXA screening also directly influence adherence to OP prevention measures.
Conclusion: To increase the adherence to recommended osteoporosis prevention in women DXA screening and educational programs are effective, and so they must be the part of preventive strategy of OP in Russia.
Disclosure: The authors declared no competing interests.

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The Inhibitory Effect of 15-Deoxy-$\Delta^{12,14}$-Prostaglandin J_2 on Bone Loss in Ovariectomised Mice
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Osteoporosis, a skeletal disorder characterized by low bone mass associated with destruction of trabecular structure, threatens bone health of women in the world. The major contributing factors of osteoporosis are withdrawal of ovarian oestrogen production in postmenopausal women. 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2), an endogenous ligand for peroxisome proliferator-activated receptor gamma (PPARγ), is one of the terminal products of the cyclooxygenase 2-catalysed reactions. 15d-PGJ_2 has been known to exert various biological actions, such as anti-inflammatory, anti-viral, anti-tumour, and apoptotic activities. Previous several studies also demonstrated that 15d-PGJ_2, as PPARγ agonist, ameliorated rheumatoid arthritis and suppressed RANKL or TNF-α-mediated osteoclast differentiation. This study investigated the activity of 15d-PGJ_2 on bone loss of postmenopausal oestrogen deficiency in ovariectomised (OVX) mice. The OVX mice causing oestrogen deprivation led to a gain of body weight, changes of bone turnover markers in blood serum, and significant destruction in femoral bone, compared with sham-operated mice. However, 15d-PGJ_2 treatment inhibited the increase of body weight, and almost recovered the serum levels of TRAP, CTX, ALP, osteocalcin and calcium in OVX mice. 15d-PGJ_2 also reduced levels of pro-inflammatory cytokines, including TNF-α and IL-1β, increased by ovariectomy. Histomorphometric and histological analysis supported that 15d-PGJ_2 blocked the damage of femur trabecular architecture in OVX mice. In particular, anti-osteoporotic effect of 15d-PGJ_2 at high dosage is similar to that of 17β-oestradiol. Taken together, these results propose that 15d-PGJ_2 may inhibit trabecular bone loss by oestrogen deficiency.
Disclosure: The authors declared no competing interests.

P376
The Impact of Multifaceted Osteoporosis Group Education on Patients Decision-Making in Regard to Treatments Option and Lifestyle Changes
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Background: For patients with chronic diseases like osteoporosis, self-care decision-making is an on-going challenge

involving medical treatment and the complexity of activities related to the patients' daily life. Disease specific group education (GE) may be one way of supporting patients in the decision-making process. This study explores multifaceted GE with particular focus on its impact on patients' decision-making in regard to both treatment options and lifestyle changes.

Methods: Fourteen women and three men diagnosed with osteoporosis participated in the study. An interpretive description design using ethnographic field study was applied. Data consisted of participant observation (78 hours) during GE and individual interview.

Result: Attending GE had an impact on participants' decision-making, because participants changed their understanding of how to implement lifestyle changes that are more beneficial to their bone health. During GE, teachers and patients expressed evidence-based knowledge and personal experiences and preferences, respectively, leading to a two-way exchange of information and deliberation about the recommendations. Even though teachers and participants explored the implications of the decisions and shared their preferences, teachers outlined that it was the participants who ultimately had to make the decision. Teachers therefore abdicated from participating in the final step of the decision-making process.

Conclusion: GE can initiate patient reflection and support decision-making. Participants decided on various steps to manage osteoporosis that they had not considered previously and made many positive decisions regarding a bone healthy lifestyle and how to implement a bone healthy lifestyle.

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P377

Factors Related to Intentional Non-Adherence to Medication in Osteoporosis Patients in Primary Care

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Background: Adherence to medication for osteoporosis is suboptimal, resulting in increased morbidity, untimely mortality and increased costs. About 50% of patients discontinue medication too early or forget to take their pills. Moreover, about 30% of patients do not even start taking medication. Little is known about these patients with so-called intentional non-adherence. The objective was to explore factors and considerations related to intentional non-adherence in patients and family physicians (FPs).

Methods: Qualitative, grounded theory. Semi-structured interviews patients who decided not to start treatment and their family physicians. Interviews were performed until saturation was reached. Two researchers independently analysed the interviews and categorised factors. Primary care setting. Patients who have been advised by their FP to take bone sparing medication after being diagnosed with osteoporosis but did not do so. FPs who have given the treatment advice were also interviewed. Inclusion criteria: willingness to participate. There were no exclusion criteria. Intervention/instrument: Semi-structured interviews. Main and secondary outcome measures: Factors related to intentional non-adherence from patients' and doctors' perspectives.

Results: 19 patients and 13 FPs were interviewed. The following main themes have been identified in patients: 1. Unwillingness to take medication to prevent age related health hazards. 2. Fear of side effects of drugs in general. 3. Mistrust in bisphosphonates. 4. Overestimation of their own health condition. 5. Underestimation of consequences of osteoporosis. 6. Perceived ambiguous communication by the FP. 7. Miscommunication or insufficient understanding of the advice given by the FP. FP results: Insufficient knowledge and unfamiliarity with the disease and its treatment to be able to motivate the patient. Reservation of the FP about bisphosphonate treatment. General animosity of the patient towards medication. Fear of side effects. Underestimation of the consequences of osteoporosis. Prioritisation by the patient.

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P378

Different Radiographic Progression of Vertebral Compression Fractures under Anti-Osteoporotic Therapies

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Background: In postmenopausal women with vertebral compression fractures (VCF), the mechanisms regulating healing processes and an anti-osteoporotic treatment are not completely clarified. The aim of this prospective study was the evaluation of bone turnover markers, bone mineral density and radiographic progression of one or more VCF during assumption of bisphosphonates, denosumab, strontium ranelate or teriparatide.

Methods: Women with recent osteoporotic VCF verified through magnetic resonance were assigned to receive bisphosphonates (BIS group, n = 28), denosumab (DMAB= 25) or strontium ranelate (SR group n= 16) or teriparatide (TPTD group, n = 24) following guidelines of Italian regulatory agency. Serum and urinary bone turnover markers and lateral thoraco-lumbar spine X-rays were obtained at 0, 3, 6, 12 months of therapy. Lumbar BMD was measured by DEXA before and 12 months after treatment initiation.

Results: At time 0 serum markers of bone formation alkaline phosphatase (ALP), osteocalcin (OC) and of bone resorption desoxipiridoline (DPD) were around higher level of normality. Between 3rd and 6th month within the consolidation process OC remain high in TPTD group while those in other groups remained significantly lower. In the same period ALP levels decreased in BIS group, unchanged in DMAB and SR groups and increased in TPTD group. DPD remain high in TPTD group; while in all groups were significantly and constantly reduced

in 6 month. Serum OPG levels remained unchanged in all antiresorptive groups while reduce in anabolic group. Lumbar BMD increased significantly at 12th month in all groups and in particular in TPTD group. An inconstant progression in VCF on radiograms were detected in first six months with BIS and SR groups.
Conclusions: In recent osteoporotic VCF a different radiographic progression with a divergence between the formation and resorption markers has been revealed under anti-osteoporotic therapies.
Disclosure: The authors declared no competing interests.

P379
Effect of N-Methyl Pyrrolidone (NMP) on Alveolar Bone and Tooth Mineralisation in Osteoporotic Induced Rat Model
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Osteoporosis is a skeletal disorder prevalent in post-menopausal women affecting bone including periodontal bone loss, tooth loss and resorption of jaw mass. It has been demonstrated that osteoporotic women are at higher risk of tooth loss and deterioration of mandibular bone. However, it remains unclear if osteoporosis affects simultaneously tooth mineralisation and bone mass. This study aimed to test the therapeutic efficacy of N-methylpyrrolidone (NMP) in preventing alveolar bone resorption and preservation of tooth integrity using the classical osteoporotic rat model established for the evaluation of enhanced osteoporosis through ovariectomy (OVX). Female Sprague-Dawley rats were randomly divided into sham-operated group (Sham) either treated or not with NMP. Bilateral ovariectomy or Sham operations were performed as previously designated and the weight of the animals was measured weekly. The rats were sacrificed after 15 weeks, the jaw bones were isolated and tooth pulp collected. Bone and tooth parameters were evaluated using X-ray radiography, immunohistochemistry, histology and scanning electron microscope. Histological results revealed that OVX incisors presented the thinner pre-dentin structures than Sham incisors and that NMP treatment normalized the pre-dentin thickness. Immunohistological staining and RT-PCR further demonstrated that odontoblasts specific protein dentin sialoprotein in the dentin pulp complex of rat incisors were significantly decreased in comparison with Sham and NMP treated group. Mandibular and teeth parameters were analysed using X-ray radiography and scanning electron microscopy confirming a significant difference between Sham, OVX NMP and OVX Veh group. It has been already established and we demonstrated here as well that estrogen deficiency causes loss of bone mass and reduces the dentinogenic capacity and tooth mineralisation. However, our results suggest that NMP has a remarkable antiosteoporotic activity preventing bone loss and minimising changes in tooth integrity making NMP a promising candidate for treatment of postmenopausal osteoporosis and other bone diseases.
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P380
There are Differences in Bone Turnover using Diverse Bisphosphonates in Daily Routine
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Background: Bisphosphonates (BPs) are the main options in medical treatment of osteoporosis. They decrease the bone resorption and bone formation. Currently alendronate (ALN), ibandronate (IBN), risedronate (RIS) and zoledronate (ZOL) are approved for the treatment of osteoporosis. It raises concerns about the dimension of decrease of bone remodelling. Therefore, we have investigated bone markers in treatment with diverse BPs in daily routine.
Methods: A retrospective monocentric study including 99 BP-naive female patients suffering postmenopausal osteoporosis was performed. The patients received either oral ALN or RIS and iv. IBN or ZOL. Measurements of bone specific alkaline phosphatase (BAP), tartrate resistant acid

Table1 [P380]:

	Aln	RIS	IBN	ZOL
number of patients (n)	22	24	25	28
age (y)	67,8±6,3	66,8±6,9	67,2± 6,7	66,2±7,3

TRAP(5b) (U/l): reduction in %:

ALN	RIS	IBN	ZOL
-7,91 ± 36,04	-2,31 ± 29,53	-7,25 ± 26,91	-6,15 ± 41,99

BAP (µg/l): reduction in %

ALN	RIS	IBN	ZOL
-14,58 ± 22,77	-8,11 ± 31,65	-25,08 ± 22,13	-3,55 ± 0,0

phosphatase (TRAP5b), calcium and phosphorus were performed at baseline and after 3 months of treatment. Bone density was measured using QCT before and after 3 years of treatment.

Results: Bone density was not significantly different using oral or i.v. BPs (differences in % from baseline oral:4,33 ± 11,31 vs. i.v.: 7,66 ±14,25). Considering the bone markers the only difference was found between IBN and ZOL for the BAP (p< 0,02)[Table1].

Conclusion: In general, all BPs supressed bone remodelling. The most impressive effect was found for IBN with a very high reduction of bone formation. ALN was seen to supress bone resorption as intensive as ZOL. The small number of patients examined might be the reason for the lack of significance. It remains to be investigated whether these differences cause different long term effects of the BPs. Should these results of daily routine taken into consideration which BP we should choose?

Disclosure: The authors declared no competing interests.

P381

Increased Calcium Fractional Absorption from Synthetic Stable Amorphous Calcium Carbonate

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Calcium supplementation is a widely recognised strategy for realising adequate calcium intake. Recent studies revealed comparable bioavailability of all available calcium salts. Freshwater crayfish rely on amorphous calcium carbonate (ACC), a thermodynamically instable and very rare biomineralised polymorph of calcium carbonate, characterised by distinctive nanometric spherules (in contrast to the microsized crystals found in other polymorphs), as the main mineral in the exoskeleton and in their temporary storage organ, the gastrolith. Inspired by the crayfish model, we have previously shown an increase in calcium bioavailability, bone absorption and retention in rats administered with synthetic stable ACC compared with crystalline calcium carbonate (CCC). Recently, we showed ACC's beneficial effects on bone loss prevention, bone formation and bone mechanical strength maintenance in an ovariectomy rat model. In this randomised double-blind crossover trial we compared the fractional calcium absorption (FCA) of ACC and CCC in 13 early postmenopausal women, using the dual stable isotope technique. The results of this study showed that FCA of ACC was 2 times greater than that of CCC on average. One subject who was administered with calcium capsules in a fasted state presented X4.6 increase in ACC/CCC relative FCA, suggesting that the unique nanometric amorphous nature of ACC makes it independent on gastric acidity for adequate absorption. This study further highlights the superior absorption of synthetic stable ACC over other commercially available calcium supplements, suggesting its preferable effectiveness for prevention of postmenopausal-related bone loss and as a potential treatment for calcium malabsorption-related disorders or conditions.

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P382

Long-Term Whole-Body Vibration Exacerbates the Bone Loss in Ovariectomised Rat Vertebra

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In recent years, several low-magnitude high-frequency whole-body vibration (WBV) platforms are available on market as it is suggested to ameliorate bone loss caused by osteoporosis. Nevertheless, no data to date have reported the long-term effect of WBV on skeleton. The studies using ovariectomised (OVX) rats are usually within 35 days. In this study, the efficacy of 12-week WBV therapy on OVX rat bone was assessed via bone mineral density and mechanical properties. Furthermore, the cellular osteoblastic activity and mRNA expression with vibration treatment were measured using novel OVX rat long bone derived osteoblastic cell culture. The result of our study demonstrates that the duration of WBV therapy matters. On 8th week of the therapy, OVX+WBV group showed significant increment of tibia bone mineral density (BMD) as compared to the non-vibrated OVX group. Nevertheless, this positive effect faded away at the 12th week. Furthermore, WBV therapy deteriorates osteoporotic vertebra BMD and strength. We have successfully established an osteoporotic osteoblast culture proliferated from osteoporotic bone explants. The difference between normal and osteoporotic osteoblast are significantly different in ALP activity, mineralisation capability and at mRNA levels, which can be utilised as a cell model for screening new treatment in future studies. However, the osteoporotic cell culture showed no significant beneficial effect on these parameters in response to vibration. To sum, our data demonstrated that WBV can be detrimental to the vertebra. The authors do recognise the difference in skeleton between rat and human. Nevertheless, the safety of using long-term WBV on osteoporotic patients should be emphasised.

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P383

Managing a Bone Healthy Lifestyle after Attending Multifaceted Osteoporosis Group Education

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Background: Patients with osteoporosis are expected to be actively involved in preventing disease development. Multifaceted osteoporosis group education (GE) is one way of providing health education to patients with osteoporosis. Little is known about how and to what extent patients with osteoporosis implement the bone healthy lifestyles that they learned by attending multifaceted GE. The purpose of this study was to investigate if and how patients implemented the learned from attending multifaceted GE into their daily lives.
Methods: Fourteen women and three men diagnosed with osteoporosis participated. An interpretive description design using ethnographic field study was applied. Data consisted of participant observation in the participants' daily lives after they had completed GE and individual interviews before and 6-9 month after GE.
Results: After attending multifaceted GE, participants experienced enhanced attention and reflections about implementing osteoporosis preventive actions. Participants who felt secure in how to act and experienced a clear need and motivation, or who could make the action into a social event, demonstrated increased implementation of the preventive action. However, social and physical concerns, together with personal needs, prevented participants from implementing preventive actions.
Conclusions: Attending multifaceted GE can support participants in general. Furthermore, the many individual solutions and sharing of knowledge can improve patients' transfer of preventive actions.
Disclosure: The authors declared no competing interests. This work was supported by the Danish National Osteoporosis Society.

P384

Clinical Results of Nonunion after Atypical Femoral Fracture

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Background: The task force of ASBMR reported the concurrent evidences regarding atypical femoral fracture (AFF) in 2010. One of them is high incidence of delayed union or nonunion up to 26% after surgical fixation. However, a clinical report after surgical treatment of nonunion is still missing. The purpose of the study was to perform a retrospective study to review the clinical results of nonunion treatment in AFF.
Methods: Nonunion developed after surgical fixation for AFF in 15 patients (17 femora) and was treated by additional intervention(s) until union. Number of subtrochanteric fracture nonunion was 10 and shaft nonunion 7. We reviewed the medical records and radiographs of these patients and compared the results of subtrochanteric nonunion with shaft nonunion.
Results: All AFF nonunion was developed in female patients. Patients of the subtrochanteric nonunion group were younger than those of the shaft nonunion group. Femoral bowing was noted in the shaft fracture group only. Eleven out of 17 cases of AFF showed lateral cortical hypertrophy at the time of initial fracture. The effect of autogenous bone graft was acceptable in both groups. Average total numbers of operations were the same in both groups.
Conclusion: Even though the incidence of nonunion after atypical femoral fracture is high compared with other low-energy trauma, a proper treatment resulted in good clinical outcomes by single intervention in most of the cases. Healing speed of this nonunion was slow and it took a long time to be united.
Disclosure: The authors declared no competing interests.

P385

Variability of Physical Performance and Risk of Fall in Elderly Women with Severe Osteoporosis and Hypovitaminosis D

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Background: This study evaluated the variability of physical performance and risk of fall in elderly women affected by severe osteoporosis and hypovitaminosis D. Falls are quite frequent in the eldest and are a key factor for morbility and mortality so that 72 % of all deceases due to falls are related to elderly people.
Methods: We have studied women $\geq$ 75. 32 were affected by severe osteoporosis with vertebral fractures (mean number 1.4) and hypovitaminosis D (mean value 25-idrossicolecalciferol 18 ng/ml). Subjects were treated with Denosumab 60 mg and calcifediol 1.5 mg. The design of the study included at T0-T24:1) spine and hip DEXA densitometry; 2) spine X-ray with morphometry; 3) Blood tests (Calcium, Vitamin D, Parathormone); 4) Short Physical Performance Battery; 5) Tinetti balance and gait Scale (TS). The Short Physical Performance Battery (SPPB) combines a balance test following 3 different positions on increasing difficulty, a 4-metre walking path and a repeated stand-up exercise from a chair. Self-sufficient elderly subject, with reduced physical performance and with a score between 5 and 9 can be considered frail and at high risk of disability. The Tinetti Scale is a predictive index for falls that catalogues subjects according to the following scores: $\leq$ 1 non–walking people; 2 < 19 walking

people with a high risk of fall; ≥ 20 walking people with a low risk of fall. Cognitive functions were assessed through MMSE whose score was adapted to age and grade of education. Comorbidity was also evaluated.

Results: At T0 we considered:1)Short Physical Performance Battery Geriatric, mean score 7 in 76.7% subjects ($p<0.05$); 2) Tinetti balance and gait scale: mean score 8 (higt risk of fall) 85.2% subjects ($p<0.5$); mean score 1 (non walking) 14.8% subjects ($p<0.5$). At T12 we evaluated: 1) Short Physical Performance Battery, mean score 9 in 63.6% subjects ($p<0.05$); 2) Tinetti balance and gait scale, mean score 14 (high risk of fall) 91.7% subjects ($p<0.5$), mean score 1 (non walking) 8.3% subjects ($p<0.5$). At T12 in all subjects we also detected no new vertebral fractures through spine X-rays and morphometry.

Conclusion: The study evaluated the incidence of vertebral fractures in the spine region in elderly women affected by severe osteoporosis and hypovitaminosis D. Since a reduced physical performance and an increase in the risk of fall indicate fraility in the elderly affected by severe osteoporosis and hypovitaminosis D, we evaluated, after Denosumab treatment , the variations in the severity markers.

Disclosure: The authors declared no competing interests.

P386

Comparison of 2 different dosing regimes 6th and 7th month interval of denosumab in post-menopausal women

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The authors observed the group of 180 post-menopausal women treated with 60 mg of denosumab applied subcutaneously every 6th (120 women) or every 7th week (60 women) for the period of 2 years. Dosing régime was selected randomly. Examination of bone markers, DXA, spineX-ray for elimination of compressive fractures, hip X-ray for evaluation of femur microarchitecture were performed at the beginning of treatment. Denosumab treatment was started in patients with -2,5 T-score in spine or hip with proven compressive fracture in the area of TH or LS or where significant decrease of bone mineral density (6 a more % yearly) was observed during previous sufficient calcium and vitamin D substitution.

Bone turnover markers were evaluated one week before next denosumab injection. BMD was measured every year and comparison was made within the same dosing régime and between the dosing régimes.

Significant decrease of PINP was indicated in the 6 months dosing group already after 6 months of treatment. Significant changes of CTX, ALP and osteocalcin were achieved after 1 year. Changes of BMD were similar after 12 months in spine in both groups (3%) and greater gain in BMD was observed after 24 month in 7 month group (6% vs 5%).

In the 6months dosing group were in first year 0% increase in total hip BMD and 4% in femoral neck in comparison with 4% and 2% in second year.

In 7 months interval we observed 1% change in first year in total hip BMD and 4% in femoral neck, in second year 3% in total hip and 2% in femoral neck.

In the 7 months dosing group bone markers changed significantly after 1 year. There were no significant changes between both groups in BMD. Both groups were same in terms of initial BMD, age and fracture risk based on FRAX.

Prolonged dosing régime of denosumab to 7 months seems to be more suitable for postmenopausal women with normal level of bone markers. Considering the long term treatment this régime might be more effective in prevention of atypical fractures or osteonecrosis of jaw while maintaining long term patient compliance.

Disclosure: The authors declared no competing interests.

P387

Sequential Treatment with Teriparatide and Denosumab in Severe Pregnancy- and Lactation-Associated Osteoporosis

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Background: Pregnancy- and lactation-associated osteoporosis (PLO) is an uncommon disease which is usually presented in the third trimester or early post-partum and the prominent clinical feature is the severe and prolonged back pain and height loss. To date the prevalence and the aetiology of this disorder are unclear and there are no guidelines for its treatment.

Methods: We report the outcomes of sequential treatment with teriparatide and denosumab in a woman suffering from severe PLO with 6 fragility vertebral fractures, severe back pain and remarkable low BMD.

Results: Breastfeeding was terminated (2 months after delivery) and treatment was started with calcium 500 mg/day, vitamin D3 2.200 IU/day and teriparatide 20 μg/day for 2 years followed by denosumab 60 mg/6 month. Shortly after the initiation of teriparatide treatment, back pain gradually decreased. One year later, the patient was almost free of back pain without new clinical vertebral fractures and her laboratory tests were normal. After 2 years of teriparatide treatment, BMD increased by 35.6% at the lumbar spine and 5.8% and 16.2% at the left total and femur neck, respectively. After one more year of demosumab treatment, BMD found to be increased from the base line values by 41.5% at the lumbar spine and 12.6% and 11.2% at the left total and femur neck, respectively.

Conclusion: Women with PLO may suffer from fragility vertebral fracture(s), often multiple, which cause severe and disabling back pain and kyphosis. Treatment with teriparatide, simultaneously with weaning, calcium and vitamin D supplementation, may increase considerably BMD, improve back pain and quality of life and prevent further occurrence of vertebral fractures. In order to preserve or further increase BMD following teriparatide treatment, sequential treatment with denosumad proved a reasonable option.

Disclosure: The authors declared no competing interests.

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Salt Osteoporosis Study, an Open Randomised Pragmatic Trial, Studying a Primary Care Structured Identification and Treatment Programme in Women Aged 65 Years or Older: Study Design and Progress

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The aim of the study is to examine whether structured identification and treatment of patients with a high fracture risk in primary care reduces fractures in comparison with usual care. An open randomised pragmatic trial using a stepwise approach in women aged 65 years or older in GP practises is being performed. Women having at least one clinical risk factor for osteoporosis will be assigned to the intervention or control group using individual randomisation. Only those women with an increased fracture risk according to an adapted FRAX in combination with DEXA and IVA will be offered bone sparing drugs. The subjects in the control group will undergo the same examinations as the intervention group at the end of the three year trial and will be classified in low and high fracture risk retrospectively. Primary outcome is time to first new fracture. A comparison will be made between women in the intervention and control group having a high fracture risk. So far n= 22791 women from 238 GP practices have been included in the study of which n= 11702 had at least one clinical risk factor for osteoporosis and were randomized. Of the n=5933 women in the intervention group, n= 1570 were classified as having high fracture risk. We shall present the design and progress of the study.
Disclosure: The authors declared no competing interests. Funded by Stichting Achmea Gezondheidszorg.

P389

Effects of Osteum, a Natural Ingredient, Containing Micellar Calcium, Vitamin D and K2, on Bone Mineral Density

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The aim of postmenopausal osteoporosis treatment is to decrease bone resorption and/or increase bone formation. Because of the slow bone turnover, osteoporosis prevention and therapies are long-lasting, implying great costs and poor compliance. Even if the effect of nutrition on bone is not as marked as that of pharmaceutical agents, it can be of great help. The nutritional intervention can be done alone preventively or as an adjuvant therapy in more severe cases. Some nutritional components such as calcium and vitamin D are recognised to have a positive effect on bone. The purpose of our study was to demonstrate the efficiency of Osteum™, a natural ingredient including micellar calcium, vitamin D and K2, to improve bone mineral density (BMD). The *in vitro* study, using primary murine bone cells, showed that Osteum™ does not modulate cell growth but that it is able to stimulate osteoblast differentiation as shown by increase ALP activity and mineralisation and to inhibit osteoclast's differentiation and resorption activity as shown by reduction of bone resorption. *In vivo*, using a model of ovariectomised mice, we showed that BMD was dose-dependently improved after Osteum™ ingestion. We also report increased osteoblast activity as shown by increase of ALP activity and decreased osteoclastogenesis as shown by reduced CTX activity. Improvement in BMD was observed after an intervention period of at least 8 weeks. This suggests that the acute effect of Osteum™ is mild but, when chronically ingested, the effect was found to be significant. Our results show that a dairy product providing not only calcium and vitamin D but also vitamin K is more efficient than calcium and vitamin D alone for BMD improvement.
Disclosure: The authors declared no competing interests.

OTHER DISEASES OF BONE AND MINERAL METABOLISM

P390 (OP38)

P391 (OP3)

P392 (OP5)

P393 (OP17)

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Non-Surgical Hypoparathyroidism in Denmark – Epidemiology, Mortality and Complications

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Background: Non-surgical hypoparathyroidism (NS-HypoPT) is a rare disease, characterised by low levels of calcium and PTH. A number of genetic variants have been shown to cause inadequate PTH secretion, although the aetiology often remains unknown. It may also appear on autoimmune basic, either isolated, as a part of the autoimmune polyendocrine syndrome, associated with APS-1, or as acquired antibodies that activate the calcium sensing receptor (CaSR). Autosomal dominant hypocalcaemia (ADH) is caused by an activating mutation (gain-of-function) in the CaSR. Little is known about this group of patients, including their mortality and morbidity. The aim was to identify all patients diagnosed with NS-HypoPT in Denmark and assess their mortality and risk of complications.
Methods: Cases (patients with NS-HypoPT) were identified through registers and review of their individual hospital charts. To access their mortality and morbidity we compared the cases with a group of age-and gender matched population based controls.
Results: In a population of 5.336.394 persons, a total of 180 cases with NS-HypoPT were identified, among whom

123 (68 %) were alive at the day of follow-up, equal to a prevalence of 2.3/100,000 inhabitants). Only 38 were genetic verified. Compared with controls, mortality was not increased, but patients had a significantly increased risk of seizures (Hazard ratio [HR] 10.05) renal insufficiency (HR 6.01), cataract (HR 4.21), neuropsychiatric complications (HR 2.45), infections (HR 1.94), cardiovascular diseases (HR 1.91) and fractures at the upper extremities (HR 1.93). In contrast, patients had significantly reduced risk of malignant diseases (HR 0.44).
Conclusion: NS-HypoPT is a rare disease associated with a number of complications that should be considered when taking care of these patients.
Disclosure: The authors declared no competing interests.

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Inhibition of TGFβ Signalling Delays Ossification in Patients with Fibrodysplasia Ossificans Progressiva
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Fibrodysplasia ossificans progressiva (FOP) is a rare congenital disorder characterised by progressive heterotopic ossification. FOP patients only present great toe malformations at birth. However, as they grow older they develop soft tissue lumps as a result of flare-ups causing the irreversible replacement of skeletal muscle tissue with bone tissue leading to cumulative physical immobility. Classical FOP patients possess a mutation (c.617G>A; R206H) in the activin receptor IA (*ACVR1*)-encoding gene which is associated with dysregulated bone morphogenetic protein (BMP) signalling. Nonetheless, not all FOP patients with this mutation exhibit equal severity in symptom presentation or disease progression, which indicates a strong contribution by environmental factors. Although inflammation is known to be the main trigger of flare-ups the molecular pathway remains largely unknown. Our objective was to study the process of osteogenic differentiation in primary dermal fibroblasts from five FOP patients based on a novel method of growth factor-induced osteogenic transdifferentiation. In all patients, the presence of the classical FOP mutation was confirmed. The osteogenic properties of the cells were evaluated by the mRNA expression of Runt-related transcription factor 2 (Runx2), alkaline phosphatase (Alp), osteocalcin (OC) and the presence of mineralization by Alizarin Red staining. Given the pro-inflammatory role of TGFβ, we also performed pharmacological inhibition of TGFβ signalling by the TGFβ type I receptor inhibitor GW788388. During osteogenic transdifferentiation the expression of Runx2 and Alp over time was higher in FOP cell lines compared to heathy controls (Runx2:p=0.001; Alp:p>0.05). All cell lines exhibited increase in mineralization. Addition of the inhibitor to the osteogenic media resulted in the attenuation of osteogenic differentiation shown by the decrease in expression of osteogenic markers in patients vs untreated cells (Runx2:p=0.045) and mineralisation. We suggest that TGFβ is involved in the molecular pathway of flare-up-induced ossification. Inhibition of this pathway may limit ectopic ossification in FOP.
Disclosure: The authors declared no competing interests.

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Short-Term Effect of Oestrogen on Human Bone Marrow Fat
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Background: Bone marrow fat is functionally distinct from subcutaneous and visceral fat depots. Bone marrow fat increases with ageing and menopause and is inversely related to bone mass. We previously observed a pronounced variation in bone marrow fat fraction during the menstrual cycle. We hypothesised that this variation is associated with changes in oestrogen levels. Our objective was to determine the effect of 17-β oestradiol administration on the vertebral bone marrow fat fraction.
Methods: We measured vertebral bone marrow fat fraction with MRI using Dixon Quantitative Chemical Shift Imaging (QCSI) every week for 6 consecutive weeks in 6 healthy postmenopausal women before, during and after two weeks of oral 17-β oestradiol administration (2 mg/day). In addition, we determined serum concentrations of bone turnover markers C-terminal crosslinking telopeptides of collagen type I (CTx) and procollagen type I N propeptide (P1NP). Data were analysed by linear mixed model.
Results: The vertebral bone marrow fat fraction decreased during two weeks of 17-β oestradiol administration ($p<0.001$) and increased after cessation. P1NP concentrations increased ($p<0.05$) and CTx concentrations decreased ($p<0.001$) during 17-β oestradiol administration.
Conclusion: We demonstrated that 17-β oestradiol administration rapidly decreases vertebral bone marrow fat fraction, suggesting that 17-β oestradiol affects bone marrow fat independent of bone mass.
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Cell Distribution of Autologous Bone Marrow Mesenchymal Stem Cells with Intra-Arterial Infusion in Dogs

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Background: The aim was to observe the distribution of autologous bone marrow mesenchymal stem cells with intra-arterial infusion in osteonecrosis of the femoral head (ONFH) in dogs.
Methods: Twelve mature male Beagle dogs were randomly divided into two groups: MSCs group and control group. ONFH models were established by a liquid nitrogen freezing method. MSCs were isolated, cultured, identified, and labelled. Three weeks after establishment of ONFH models, intra-arterial infusion was performed. 6 dogs in MSCs group were injected with 1 ml MSCs (5X106–1X107/ml), while 0.9% normal saline was used in control group. The dogs were sacrificed at eight weeks after intra-arterial infusion. Changes in general architecture of the femoral heads were determined by hematoxylin and eosin stain. The differentiation of grafted cells in the femoral heads was observed by immunofluorescence, and distributions of grafted cells in vital organs were observed through immunohistochemistry (IHC) and histomorphometry.
Results: Trabecular bone volume was increased and empty lacunae rate was decreased in MSCs group ($p<0.05$). Most of the BrdU-positive MSCs in necrotic region of MSC-transplanted femoral heads co-stained together with osteocalcin. Simultaneously, the BrdU-positive MSCs were nonuniformly distributed in vital organs and the number of BrdU-positive cells in kidney (IOD/Area=0.050±0.0013), gallbladder (IOD/Area=0.032±0.0020) and liver (IOD/Area=0.023±0.0026) were obviously higher than those in small bowel (IOD/Area=0.005±0.0009), prostate (IOD/Area=0.005±0.0006), testicle (IOD/Area=0.003±0.0004) and spleen (IOD/Area=0.003±0.0002). Furthermore, no immunological rejection and graft versus host disease appeared in dogs after transplantation.
Conclusions: MSCs with intra-arterial infusion can migrate into the necrotic field of femoral head and directionally differentiate into osteoblasts, and then improve repair of femoral head. Furthermore, MSCs were unequally distributed in vital organs but no conflict with physical condition could appear. Autologous bone marrow MSCs with intra-arterial infusion may be a feasible and safe avenue for the treatment of femoral head necrosis.
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Altered Osteocyte Lacunae Characteristics in Brtl/+ Mice: a Model for Osteogenesis Imperfecta Type IV

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Osteogenesis imperfecta (OI) is a genetic disorder caused by mutations in genes encoding type I collagen or collagen-interacting proteins. The Brtl/+ mouse is a model for dominant type IV OI, with a Gly349Cys substitution in one *COL1A1* allele resulting in long bone fragility. Bone material quality is known to be altered in OI. However little is known about the impact of OI on osteocyte lacunae. We investigated osteocyte lacunar density, size and shape using 2D-image analyses of sectioned Brtl/+ cortical bone. Transverse (wild-type n=6; Brtl/+ n=6) and longitudinal (wild-type n=10; Brtl/+ n=8) femoral midshaft sections from 2-month old mice were acquired by quantitative Backscattered Electron Imaging. The image grey-levels were thresholded at a value corresponding to 5.2 weight% calcium content. We obtained binary images of sectioned osteocyte lacunae areas (OL) in the range of 1.55µm² to 80µm². We measured the OL density, OL-related porosity and frequency distribution of OL area, perimeter and aspect ratio between major and minor axes (AR). Neither OL density nor porosity were significantly different between wild-type and Brtl/+ in transverse and longitudinal sections. Independent of section orientation, the size distribution of lacunae showed decreased heterogeneity in Brtl/+, with higher numbers of intermediate OL and lower numbers of small and large OL per surface in Brtl/+ vs WT. For instance, in the frequency distribution of OL area in transverse sections, Brtl/+ *vs* wild-type -18% in the 1.55 to 10 µm² range, +23% in the 10 to 20 µm² range and -32% in the 60 to 70µm² range. Additionally, the OL shape in Brtl/+ were significantly more elongated, consistent with lacunar size distribution and with detection of more lacunae in transverse than longitudinal sections. These data suggest that osteocyte lacunar formation is altered in OI, which could be secondary to either osteocyte dysfunction or mutant collagen structure.
Disclosure: The authors declared no competing interests.

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Maternal TRPV6 is Crucial for Bone Development

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Calcium is essential for many physiological processes, including cell signalling, nerve excitation, but also skeletal mineralisation. Despite expression of the calcium channel TRPV6 in bone cells, young mice defective for the calcium-transporting

pore region of TRPV6 (TRPV6^{D541A}) do not develop a bone phenotype. Since TRPV6 is highly expressed in the placenta, we assessed whether placental TRPV6 deficiency, known to disturb calcium homeostasis in foetal development, affects bone development. Besides, we assessed the effect of low calcium diets on bone development of these mice. Homozygous TRPV6^{D541A} mothers that are TRPV6 deficient were crossed with heterozygous fathers, yielding male homozygous TRPV6^{D541A} offspring. Directly after weaning, they were put on a normal (1% w/w) or low (0.2% w/w) calcium diet for 6 weeks. Bone microarchitecture and strength were assessed at 9 weeks, using microCT and 3-point bending tests. TRPV6^{D541A} offspring from homozygous mothers displayed reduced cortical bone mass when compared with wildtype mice (cortical volume (Ct.V),-15%, perimeter,-10%, moment of inertia (MOI),-30%). When these mice were put on a low calcium diet, both trabecular (BV/TV,-50%, trabecular thickness (Tb.th),-25%, Tb.N,-40%) and cortical (Ct.V,-40%, perimeter,-20%, MOI,-60%) bone mass were severely diminished along with compromised bone strength (work-to-failure,-50%) and reduced mineral bone formation rate (MAR and BFR,-65%) as assessed by histomorphometry. TRPV6^{D541A} offspring from heterozygous mothers (exposed to one functional placental TRPV6 allele) on a low calcium (0.2% w/w) diet had reduced cortical bone mass compared to wildtype littermates (Ct.V,-20%, perimeter,-10%, MOI,-30%). The current study demonstrated that placental TPRV6 is essential for normal cortical bone development. Additional comparative neonatal calcium diet studies with homozygous and heterozygous TRPV6 deficient offspring uncovered that TRPV6 is critical for proper trabecular bone formation and to limit detrimental effects of low calcium on cortical bone. Overall, this study demonstrates the importance of TRPV6 for bone in case of low calcium stress.

Disclosure: The authors declared no competing interests.

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Evaluation of *SQSTM1* Missense Variants of Unknown Clinical Significance in Paget's Disease of Bone

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Determining the importance of coding variants is an increasing challenge in human molecular genetics, exemplified in the skeletal disorder Paget's disease of bone (PDB) where mutations affecting *SQSTM1* are commonly identified. As part of a screening effort to enter asymptomatic adults carrying *SQSTM1* mutations with a family history of PDB into the Zoledronate in Prevention of Paget's disease (ZIPP) study, exons 7 and 8 of the gene were sequenced in 1420 probands with PDB. Subsequent sequencing of first degree relatives (predominantly children) of probands found to be positive for *SQSTM1* mutations led to the identification of 324 asymptomatic subjects (44%) with mutations, who were invited to enter the trial. The analyses identified one family in the UK carrying a novel Ala390Gly missense mutation and one family in Australia with an Ala390Val mutation, located close to the ubiquitin-binding UBA domain of the SQSTM1/p62 protein. Although mutation pathogenicity prediction programmes indicated both variants may be pathogenic, analysis of protein structural models showed that Ala390 is not directly involved in either dimerisation or ubiquitin-binding of the UBA domain, properties critical to protein function. Protein binding assays showed the Ala390Val variant, but not Ala390Gly, is however associated with a loss of ubiquitin-binding function, which for Ala390Val mirrors that seen for pathogenic *SQSTM1* mutations. In contrast, cell-based reporter assays showed both variants strongly activated NF-kB signalling relative to wild-type SQSTM1/p62, indicating both are probably pathogenic. On the basis of the functional analyses the Ala390Val and Ala390Gly positive subjects received genetic counselling to the effect that they were carriers of pathogenic mutations and were offered the chance of participating in the ZIPP study. Our work highlights a disconnect between *in silico* predictions, protein function analyses and cell-based assays of *SQSTM1* sequence variants, and suggest cell-based assays are required to identify those variants which are potentially pathogenic.

Disclosure: The authors declared no competing interests.

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Plasma N-3 Polyunsaturated Fatty Acids are Positively Associated with Bone Density in Renal Transplant Recipients

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Background: Marine n-3 polyunsaturated fatty acids (n-3 PUFA) may have beneficial effects on bone. Dietary intake of marine n-3 PUFA, found mainly in fish and seafood, has previously been found positively associated with bone density in otherwise healthy subjects. Renal transplant recipients (RTR) suffer high rates of bone loss and increased risk of fractures. The aim of this study was to investigate the association between plasma marine n-3 PUFA-levels and bone mineral density (BMD) in a large cohort of RTR.

Methods: A total of 701 RTR were included in this cross-sectional analysis. BMD of lumbar spine, proximal femur and

distal forearm were measured by dual energy X-ray absorptiometry (DEXA)-scan, and blood samples were drawn in the fasting state 10 weeks post-transplant. Multiple linear regression analysis was used to assess the association between n-3 PUFA and BMD.
Results: Median age was 53.9 years and two thirds were men. Based on femoral neck T-scores, 25% of patients were osteoporotic, and 50% osteopenic. Significant positive associations were observed between plasma marine n-3 PUFA and BMD at total hip (β = 0.0041, p = 0.02) and lumbar spine (β = 0.0058, p = 0.02) after multivariate adjustment. No association was found between n-3 PUFA and BMD of the distal forearm, while at the femoral neck the association was only significant in age and gender adjusted analysis.
Conclusion: We found positive associations between plasma marine n-3 PUFA-levels and BMD at the total hip and lumbar spine 10 weeks post-transplant. Marine n-3 PUFA may represent a potential to improve bone disease after renal transplantation.
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Mouse with Substitution of Type I Collagen 3-Hydroxylation Site has Altered ECM but does not Recapitulate the Bone Dysplasia of Types VII/VIII Osteogenesis Imperfecta

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Recessive types VII and VIII osteogenesis imperfecta (OI) are severe bone dysplasias caused by null mutations in prolyl 3-hydroxylase 1 (*P3H1*) or cartilage-associated protein (*CRTAP*), two mutually supportive components of the prolyl 3-hydroxylase complex expressed in bone and cartilage. Individuals with types VII/VIII OI are missing the complex components as well as 3-hydroxylation of the collagen substrate residue α1(I)P986. This primary modification defect leads to increased post-translational lysine hydroxylation of the type I collagen helix, consistent with delayed folding. Since α1(I)P986 has been proposed to play a role in fine-tuning the alignment of collagen helices within fibrils, we sought to clarify the role of the P986 substrate modification in bone dysplasia and collagen overmodification by generating a knock-in mouse model with an α1(I)P986A substitution that cannot be 3-hydroxylated. We verified exclusive expression of α1(I)A986 collagen in murine tissues and cellular cDNA and protein. Neither heterozygous ($986^{P/A}$) nor homozygous ($986^{A/A}$) mice recapitulate critical features of types VII/VIII OI. Mutant mice had normal growth rates, femoral biomechanical properties (stiffness, ultimate load, brittleness) and collagen folding kinetics. However, the P986A substitution affected collagen biochemistry and higher order structures. Skeletal staining and radiographs revealed flared rib cages and delayed calvarial mineralisation in $986^{A/A}$ pups, with kyphosis by 2 months. Despite normal 3-hydroxylase complex levels, $986^{P/A}$ and $986^{A/A}$ osteoblast type I collagen was moderately overmodified on PAGE. Dermal fibrils of $986^{A/A}$ displayed decreased diameters and heterogeneity. Interestingly, although femoral aBMD and TMD was reduced in $986^{A/A}$ versus WT, bone matrix mineralisation density distribution assessed by qBEI was normal. Together, these data suggest that 3-hydroxylation of α1(I)P986 is important for regulating type I collagen modification, crosslinking and mineral organisation in bone, but does not cause the severe bone pathology of collagen 3-hydroxylation defects, which likely result from absence of the ER complex and cartilage α1(II)P986 3-hydroxylation.
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Pseudohypoparathyroidism in Denmark – Epidemiology, Morbidity And Mortality

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Background: Pseudohypoparathyroidism (Ps-HypoPT) is caused by an inactivating mutation in the GNAS1 gene located on chromosome 20q13. It is characterised by target organ resistance to PTH, resulting in hypocalcaemia and hyperphosphataemia. Ps-HypoPT is often subdivided into different types, which can be distinguished by clinical and hormonal findings. Furthermore the parental inheritance is of importance. Studies on this group of patients are very sparse. The aim of the study was to identify all Danish patients with Ps-HypoPT and access their mortality and morbidity.
Methods: Patients with Ps-HypoPT were identified through the Danish National Patient Registry and the prescription database, with subsequent validation of their individual hospital chart. For each identified case three age- and gender matched controls were randomly extracted from the background population.
Results: We identified a total of 60 cases, equal to a prevalence of 1.1/100.000 inhabitants, 42 were women. Only 23 % (14 persons) have an identified mutation in the GNAS gene. Compared with controls, cases have a significantly increased risk of cataract ($p<0.01$), seizures ($p>0.01$), neuropsychiatric disorders ($p<0.01$) and infections ($p<0.01$). However, mortality, risk of renal disorders, cardiovascular – and malignant diseases and fractures were compatible to the general

background population. A subgroup analysis on the genetically verified cases showed the same tendencies.
Conclusion: Ps-HypoPT is a rare disease associated with a number of complications, that should be considered when taking care of the group of patients.
Disclosure: The authors declared no competing interests.

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Elevated Osterix Expression Enhanced FGF-23 Secretion from Causative Tumours of Oncogenic Osteomalacia
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Background: Oncogenic osteomalacia (OOM), or tumour induced osteomalacia (TIO), is a rare disease characterised by renal phosphate wasting and hypophosphataemic osteomalacia due to the secretion of fibroblast growth factor 23 (FGF-23) from causative mesenchymal OOM tumours, which is physiologically secreted from osteocytes. To determine whether OOM tumours have osteogenic characteristics, the expressions of osteoblast/osteocyte specific genes in OOM tumours were investigated at the transcriptional and translational levels.
Methods: Sixteen causative OOM tumours and 7 histopathologic classification-matched non-OOM tumours were analysed by quantitative real-time RT-PCR and immunohistochemistry. Fluorescent immunohistochemistry was also applied to investigate co-localisation of the gene expressions in OOM tumours. The study was approved by the institutional ethics committees and was conducted in accordance with the principles of the Declaration of Helsinki. UMR106 osteoblastic cell line was analysed to identify the role of osteocyte/osteoblast specific genes in FGF-23 expression *in vitro*.
Results: Osteocyte/osteoblast specific genes such as osterix (OSX), osteocalcin (BGP), and DMP-1 were significantly elevated as well as FGF-23 in OOM tumours compared with non-OOM tumours. The elevated expressions of these genes were also confirmed by immunhistochemistry. Fluorescent immunohistochemistry revealed that localisations of these gene expressions were merged in some OOM tumours, however, the other OOM tumours exhibited different co-localisations of OSX and FGF-23, from those of osteocalcin and DMP-1. Gene knockdown analyses using siRNA of each genes in UMR106 cells revealed that FGF-23 expression was elevated by OSX, but decreased by DMP-1. BGP decreased DMP-1 expression, but OSX had no effect on BGP or DMP-1 expressions.
Conclusion: OOM tumours have osteogenic characteristics. Different co-localisation of osteocyte/osteoblast specific genes and independent signal pathways of OSX to FGF-23 from BGP to DMP-1 suggests overexpression of OSX is one of a cause of FGF-23 secretion from OOM tumours.
Disclosure: The authors declared no competing interests.

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Specific MicroRNAs as Biomarkers in CKD Patients at High Risk for Calcifications and ROD?
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Background: Calcification of vessels, mainly in the tunica media, with concomitant demineralisation of bone is typical for patients suffering from chronic kidney disease (CKD). In this project, we analyse samples from CKD patients with a focus on microRNAs (miRNAs) as new biomarkers for vascular calcification. Our aim is to find a pattern of miRNAs indicating vascular calcification and/or mineralisation changes in the course of the disease.
Methods: Serum and plasma samples of 73 patients in CKD stages 3 – 5, 67 post RT (renal transplantation) patients as well as 36 healthy controls are analysed in the study. Additional 25 patients in CKD stage 5 were prospectively followed before, and already some of them during and after RT. Known biomarkers for calcification have been measured using ELISA techniques. A miRNA profile of CKD patients compared to healthy controls has been established using an nCounter® miRNA Expression Assay. Deregulated miRNAs were further analysed in qPCR experiments.
Results: PTH, FGF23 and osteocalcin were significantly increased in late stage CKD patients. When analysing more than 800 miRNAs, a significant difference was found in 37 when comparing CKD patients of stage 5 and healthy controls. Those miRNAs were connected to vascular smooth muscle cell (VSMC) biology and bone metabolism, especially miR-146a, miR-145 and miR-223, which showed an up to 13-fold expression difference during qPCR experiments.
Discussion: MiRNAs with a biological association to vascular calcification, bone metabolism or differentiation of VSMCs to osteoblast-like cells are deregulated to a considerable amount in CKD patients. These pathways may be important during the development of calcified tissue in the course of kidney disease. MiRNA profiles could be early diagnostic markers indicating the risk of vascular calcification or bone demineralisation in this high risk group.
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Altered Cortical Macroporosity in High Fat Diet-Induced Type 2 Diabetic Mice Revealed by Nanofocus X-Ray Computed Tomography

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Type 2 diabetes mellitus is associated with increased bone fragility and fracture risk. However, diabetes-associated alterations in structural properties of the bone remain unclear. The current study aims at characterising the cortical bone macroporosity by using nanofocus X-ray computed tomography (nanoCT) imaging. Additionally, the T2DM cancellous bone architecture was assessed. Eight-week-old male C57BL/6J mice received a high-fat diet for 14 weeks (HFD, 60% kcal from fat) and were compared with age-matched control animals (10% kcal from fat).Tibiae (n=7 and n=8 for HFD and control respectively) were scanned in fast scan mode at 2 µm isotropic voxel size with X-ray source power of 85 kV and 99 µA, 1mm Al filter, resulting in a 20 minutes scan time (Nanotom S). The images were reconstructed using phoenix datos|x 2 and analysed using CTAn (Bruker microCT). Statistical significance ($p<0.05$) was determined by a two sample Students t-test after assessing the homogeneity of variances. Midshaft tibia intracortical macroporosity was found to be significantly decreased in HDF mice compared with age-matched control ($p=0.02$; 0.29±0.05% *vs* 0.59±0.09%). Cortical thickness showed a statistical significant increase (+23 %) in HFD mice compared to controls (266.4±3.8 µm *vs* 215.4±4.0 µm; $p<0.001$). With regard to the cancellous bone, the trabecular number was decreased (-29%) while the trabecular thickness and trabecular separation were increased in HFD mice (+26% and +10.9% respectively compared to controls; $p<0.001$ and $p=0.002$, respectively), resulting in an unaltered trabecular bone volume fraction between HFD and control groups. Insights into the bone microstructure by using nanoCT revealed HFD-related alterations in both cortical and trabecular compartments. In particular, a decrease in the cortical macroporosity was evidenced. Further characterisation of the cortical porosity is in progress by investigation at 1 µm voxel size in order to extract the cortical bone osteocyte network and separate it from the vascular porosity.

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Low Sclerostin levels in long-term remission of acromegaly: an adaptive response to long-term exposure of osteocytes to high IGF-1 levels before successful treatment?

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Introduction: An increase in bone turnover is observed in active acromegaly which is due to the anabolic effect of high levels of GH and IGF-1. This decreases after normalization of IGF-1 levels. Sclerostin is an osteocyte-produced key negative regulator of bone formation, which may play a role in the persistence of bone mass after successful treatment of acromegaly, however currently no data are available.
Objective: To study the potential role of sclerostin in the maintenance of high/normal bone mass in patients in long-term remission after successful treatment of acromegaly.
Methods: Sclerostin was measured in the stored sera of 79 patients, mean age 58.9±11.5 years,49% female, in long-term remission from acromegaly, mean time of remission14.6±5.9 years. Data were compared to sclerostin levels measured in 91 healthy controls (mean age 51.1±16.9years; 59% female). BMD measurements were available in all acromegaly patients.
Results: Median plasma sclerostin was significantly lower in acromegaly patients compared to healthy controls respectively 118.3pg/ml±37.8pg/ml vs. 147.3±54.9pg/ml. This remained after adjusting for age, sex and BMI (adjusted Beta 37.8(95%CI 22.0-53.5,$p<0.001$). Mean BMD was in the high normal range at both lumbar spine(LS) and femoral neck(FN) sites. We found no significant relationship between plasma sclerostin levels and age, BMI, current IGF-1 levels or current BMD in the acromegaly patients.
Conclusion: Here we demonstrate that mean circulating sclerostin levels are lower in patients in long-term remission of acromegaly compared to healthy controls. This suggests a potentially permanent alteration in osteocyte function, resulting in impaired sclerostin production possibly resulting from the long-term exposure of osteocytes to high circulating levels of IGF-1 in the period of active acromegaly.

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The Effects of Human Periodontal Ligament Cells to Glabridin

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Periodontitis is a chronic inflammatory disease and induces periodontal tissue destruction. Periodontal ligament cell-based treatment is considered as one of the most promising methods in periodontal tissue regeneration. This study examined the regenerative effects and anti-destructive effects of glabridin on periodontal ligament fibroblasts (PDLFs). We assessed the regenerative effects of glabridin on PDLFs by measuring the expression of alkaline phosphatase, type I collagen, osteocalcin, Runx2 and osteoprotegerin using

quantitative real-time PCR. We also determined the anti-destructive effects of glabridin on PDLFs under inflammatory conditions by examining the expression of proteolytic enzymes, including matrix metalloproteinase (MMP)-1, MMP-2 and MMP-8 using quantitative real-time PCR. Additionally, we evaluated the effects of glabridin on inflammatory mediators by measuring the secretion of interleukin -1β (IL-1β), tumour necrosis factor-α (TNF-α), interleukin-6 (IL-6) and nitric oxide (NO) on RAW264.7 cells. Glabridin increased significantly alkaline phosphatase, Runx2 and osteoprotegerin mRNA expression. Glabridin suppressed the expression of MMP-1 and MMP-8 in the PDLFs. In addition, glabridin had no effect on viability of the RAW264.7 cells and decreased the release of LPS-induced IL-1β, TNF-α, IL-6 and NO in RAW264.7 cells. These findings suggest that glabridin can stimulate the osteogenic differentiation and alleviates the tissue-destructive processes that occur during periodontal inflammation.

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Impaired Intestinal Calcium Absorption and Bone Formation in Male and Female Beta-Thalassemic Mice

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A decrease in beta-globin protein synthesis leads to a hereditary anaemia known as beta-thalassemia. In addition to haematological symptoms, beta-thalassemic patients and rodents usually manifest aberrant bone metabolism and growth retardation, but the underlying mechanism remains elusive. Here we demonstrated in hemizygous beta-globin knockout mice (BKO) that beta-thalassemia was associated with a marked decrease in the duodenal calcium absorption, which could be alleviated by long-term 1,25-dihydroxyvitamin D3 supplementation. Further investigation of bone mineral density in two beta-thalassemic mouse models, namely BKO and beta-IVSII654 knockin with abnormal splicing of beta-globin gene, showed that beta-thalassemia led to low bone mineral density, as determined by *ex vivo* dual-energy X-ray absorptiometry and computed tomography, in sex- and age-dependent manner. Specifically, the thalassemia-associated osteopenia appeared to be more severe in female mice than male mice. Bone histomorphometric analysis in male beta-IVSII654 thalassemic mice revealed a marked decrease in bone formation rate with modest change in osteoclast-mediated bone resorption (as indicated by osteoclast surface and eroded surface), whereas both decreased bone formation and accelerated bone resorption contributed to osteopenia in female beta-IVSII654 mice. It could be concluded that beta-thalassemia-associated osteopenia resulted, in part, from the impaired duodenal calcium absorption and bone formation as well as a sex-dependent increase in bone resorption. This study has been approved by the animal ethics committee of Faculty of Science, Mahidol University.

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Voluntary wheel running exercise alleviates stress-induced bone loss in male rats

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Chronic stress can cause psychiatric disorders, e.g., anxiety and depression, and has been shown to induce bone loss in humans and rodents, all of which are intractable to conventional anxiolytic drugs. Regular exercise has been reported to alleviate chronic stress and enhance bone gain in non-stressed rodents, but forced exercise can aggravate stress, thereby increasing bone loss in stressed individuals. We, therefore, hypothesised that voluntary exercise, such as voluntary running in a wheel with minimal physical stress due to forced training, could alleviate bone loss after exposure to 4-week restraint stress. Herein, male rats were divided into 4 groups, i.e., non-stressed sedentary controls, non-stressed rats subjected to 4-week wheel running exercise, sedentary stressed rats, and stressed rats subjected to wheel running exercise. After the 4-week experiment, tibiae were collected for bone microstructural analyses by using micro-computed tomography (micro-CT) and bone histomorphometry. Heart and adrenal weights were recorded to confirm successful exercise and stress induction protocols. This study has been approved by the animal ethics committee of the Faculty of Medicine, Thammasat University. The micro-CT results showed that stressed rats exhibited lower trabecular mineral density than control rats with no change in moment of inertia (MMI). Voluntary running exercise significantly increased polar MMI and MMI in the y-axis in stressed rats as compared with sedentary stressed rats, suggesting that voluntary running increased bone strength. In bone histomorphometric study, stressed rats subjected to voluntary wheel running tended to have greater bone volume fraction (bone volume normalised by tissue volume; BV/TV), and had greater osteoblast surface without changes in osteoclast surface or eroded surface. It could be concluded that long-term exposure to repetitive stress deteriorated bone microstructure, while voluntary running exercise could alleviate stress-induced bone loss in male rats, presumably by inducing bone formation rather

than diminishing osteoclast-mediated bone resorption.
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P412
Calcification in the Vessel Wall – a Role of Enzymes of the Vitamin K Cycle?
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Pathophysiological calcification in the vasculature favours cardio- and cerebrovascular diseases (CVD). In patients with chronic kidney disease, vitamin K metabolites, particularly K1 and MK-4, are associated with decreased vascular calcification. We investigated the expression of components of the vitamin K cycle (VKC) and the MK-4 synthesis (MKS) in aorta and bone of 26 brain dead organ donors to identify differences in expression pattern during atherosclerosis stages in aortic vascular tissue and to compare these profiles in both tissue types. Predesigned TaqMan gene expression assays were used on a LC480 system to determine gene expression in both tissue types. Determination of calcification stages was done histologically: (0 [unaffected vessels], 1 [intima thickening], 2 [intima calcification]). Both the VKC enzymes (VKOR, VKORL1, GGCX, the chaperone calu) and the enzymes necessary for MKS (NQO1 and UBIAD1) are expressed in aortic and bone tissue. In the aorta, gene expression of VKOR, VKORL1, and calu differed significantly among the three atherosclerotic stages (p=0.040; p=0.023 and p=0.038, respectively), whereas the expression of GGCX showed only borderline significance (p=0.060) between the three stages. In bone, gene expression of VKC and the MKS proteins did not differ in the three AS stages. Comparison of bone and aorta showed only significant differences in gene expression of calu, GGCX and NQO1 in stage 2. We showed that bone and aorta express the components of MK-4 synthesis as well as the vitamin K cycle. Furthermore, we could demonstrate that a different gene expression pattern exists in AS progression in bone and aorta. These data might shed light on the role of vitamin K metabolising enzymes in vascular calcification.
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Aluminum and Iron are Deposited in the Calcified Matrix of Bone Exostoses
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Background: Exostosis (or osteochondroma) is the most common benign bone tumour encountered in children and adults. Exostoses may occur as solitary or multiple tumours (in the autosomal syndromes of hereditary multiple exostoses). Exostoses are composed of cortical and medullary bone covered by an overlying hyaline cartilage cap. The pathophysiology of isolated exostosis in unknown but multiple tumours are associated with an autosomal dominant syndrome- hereditary multiple exostoses (HME) - due to mutations in genes responsible for heparan sulfate (HS) biosynthesis.
Methods: The aims of the present study were to analyse the matrix composition of 15 of these benign tumours by histological and histochemical methods. Undecalcified sections were stained by Goldner's trichrome for osteoid, toluidine blue for glycosaminoglycans, Perls (for iron) and mordant blue (for aluminium). Specificity of the metal staining was obtained by scanning electron microscopy with energy dispersive analysis (EDX) and wavelength-dispersive spectroscopy (WDS) on the polished blocs.
Results: Histochemistry identified Al in all tumours as linear bands deposited by the osteoblasts in the calcified matrix of the trabeculae, in some areas of calcified cartilage or the cortical shell. Iron was detected in 10 out of the 15 patients as linear bands in the same locations. Osteoid thickness was normal in all patients. EDX failed to identified iron or aluminium in all the samples. WDS confirmed that the metal ions were present a very low concentration (iron: 30-495 x 10^{-6} % and aluminium 15-27 x 10^{-6} %).
Conclusions: Aluminium and iron are two metals actively substituted to calcium in hydroxyapatite crystals of the bone matrix. Histochemical analysis of iron and aluminium represents a very sensitive method. EDX having a sensitivity of 5% failed to identify these two metals in the exostoses. WDS confirmed the presence of these metals and also showed that their concentrations are were limited. Aluminium and iron are known to strongly inhibit mineralisation of bone but the osteoid thickness was normal in all cases. The presence of these two metals in the calcified matrix of bone advocates for a disturbed metabolism of osteoblasts and chondrocytes in exostosis similar to that observed in case of hemochromatosis.
Disclosure: The authors declared no competing interests.

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In Vivo And _In Vitro_ Evidence of Enhanced Immunoglobulin Production in Presence of _SQSTM1_ Mutation

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Mutations in *SQSTM1* gene encoding p62 have been associated with Paget's disease of bone (PDB) in up to 50% and 15% of familial and sporadic cases, respectively. Among other functions, p62 is the prototypical cargo receptor for autophagy, a lysosomal recycling process recently implicated in plasma cell ontogenesis and immunoglobulin (Ig) production. Apart from one report of elevated Ig levels in a subset of PDB patients, whether antibody immunity is altered in PDB has not been explored. To address this issue, we measured serum Ig levels in a cohort of 154 patients with active PDB in relation to *SQSTM1* mutation status. Compared with age-matched controls (n=60), PDB cases showed higher IgG ($p<0.05$) and gamma globulin levels by protein electrophoresis ($p<0.05$). This effect was mainly driven by patients with *SQSTM1* mutations (n=27) and became non-significant when patients without *SQSTM1* mutation were considered. A similar but not significant trend was observed in IgM levels in *SQSTM1* mutated patients versus controls (p=0.09). Moreover, among *SQSTM1* mutation carriers, patients with truncating mutations (Y383X and E396X) were associated with the highest serum IgG levels. Since treatment with nitrogen containing bisphosphonates may exert adjuvant, immune-modulating effects, Ig levels were monitored in 10 and 15 patients with and without *SQSTM1* mutations, respectively, before and after 1, 3, 6, and 12 months from intravenous treatment with zoledronic acid. In both treatment groups, IgG and IgM levels increased between 1 and 3 months from treatment and then decreased towards pretreatment levels. At each time-point, IgG levels were higher in *SQSTM1* mutation carriers than in patients without mutation. To conclusively assess whether *SQSTM1* mutations affect plasma cell activity in a cell-autonomous fashion, we lentivirally engineered the IgM-producing B18 plasmocytoma and the IgG-producing OKT3 hybridoma cell lines to stably express wild type and mutant (E396X) p62. Standard 4 hr Ig secretion assays demonstrated increased rates of Ig secretion in cell lines expressing mutant p62, as compared with controls engineered to express wild type p62. In conclusion, our clinical and experimental data indicate that PDB-associated *SQSTM1* mutations affect plasma cell activity and humoral immunity. The possible implications of these findings in the pathogenesis of the disorder and the occurrence of comorbidities in mutated PDB patients warrant further investigation.

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Vitamin D Status and Skin Damage in a Sunny Climate: Evaluation in Healthy Young Women

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A healthy balance between vitamin D status and skin safety may be difficult to achieve in sunny climates. This is particularly important in young people who may suffer serious long term consequences from either vitamin D deficiency or excessive sun exposure. We recruited 262 healthy Australian women age 16 – 25 years (mean ± SD 22.3 ± 1.2) via Facebook advertising and obtained extensive questionnaire data, UV exposure by calibrated dosimeter use, serum 25 hydroxyvitamin D (25 OHD) levels (Abbott Architect assay), Fitzpatrick skin type (higher score reflecting lesser sun sensitivity), skin melanin density (spectrophotometry of the inner upper arm) and actinic skin damage by silicone skin cast score (Beagley-Gibson grading). Mean serum 25 OHD was 60.7 nmol/L (range 20 – 201; 39% ≤ 50; n = 186). Vitamin D status was inversely related to Fitzpatrick skin type (adjusted odds ratio [OR] 0.5, P = 0.015), melanin density (adjusted OR 0.7, P = 0.045), use of summer sun protective measures (adjusted OR 0.8, P = 0.037), and positively associated with regular multivitamin use (adjusted OR 1.6, P = 0.04). Of concern, median skin cast score was 3/6 (interquartile range [IQR] 2), and 4/6 (IQR 2) in 23-25 year-olds. Skin cast score was positively associated with recorded UV exposure after adjustment for Fitzpatrick skin type, age and daily smoking ($p<0.01$). These data indicate a high prevalence of moderate-to-severe actinic skin damage in young Australian women despite high prevalence vitamin D insufficiency. Moreover, several factors that reduce skin damage and skin cancer risk were shown to be associated with lower 25OHD levels. The findings raise concern whether it is feasible to maintain adequate vitamin D status while minimising skin ageing and skin cancer risk under climatic conditions of plentiful UV availability. Carefully-designed prospective studies and clinical trials are required to address this important problem.

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PPARα is a Novel Regulator of Bone Density during Ageing

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Skeletal ageing is governed by bone cell metabolism. Common factors may exist linking cellular energy metabolism with lifespan and skeletal heath, such as those targeted by anti-diabetic drugs metformin and fenofibrate. We have used cellular and molecular studies, in combination with genetically modified mouse models to investigate the effect and mechanism of such systems on the skeleton. Pre-osteoblasts (2T3) were differentiated *in vitro* for 12 days in osteogenic media, in the presence of fenofibrate (5-25μM), metformin (5-500 μM) or a small molecule activator of the adiponectin receptors (AdipoRON, 5-25μM). Metformin significantly reduced metabolic activity and cell viability (MTS & alamar blue assays) of differentiated 2T3 cells. 2T3 mineralisation (Alizarin Red staining) was not affected by metformin, yet mineralised particle number and total mineralisation area were significantly increased by AdipoRON (4.6 and 6.0-fold, respectively, $p<0.01$) and fenofibrate treatments (8.9 and 10.0-fold respectively, $p<0.001$) relative to vehicle controls. Fenofibrate and adiponectin signalling directly activate the nuclear ligand receptor PPARα, which regulates fatty acid catabolism and transport, and interacts with known ageing-related pathways. We hypothesised that PPARα may act as a master regulator of the skeleton and mediate age-related changes in bone *in vivo*. This was tested by analysing the skeletal phenotype of middle (~7 months) and old-aged (~15 months) PPARα$^{-/-}$ or wildtype control mice using micro-CT and histomorphometric analysis. Cortical density was significantly decreased in PPARα$^{-/-}$ relative to control mice at both ages ($p<0.001$), with specific low density areas of cortical bone identified with large cartilaginous remnants. PPARα$^{-/-}$ trabecular BV/TV was not different to controls at middle age. However, unlike the BV/TV of wildtype mice, PPARα$^{-/-}$ BV/TV did not decrease with further ageing. Adiponectin signalling may have a beneficial effect on bone formation through PPARα, raising a putative novel use for the fibrate class of PPARα-activators for improving bone quality with ageing.

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Using a Novel Tissue Engineered Skeletal Muscle Model to study the Pathogenic Mechanism of Heterotopic Ossification

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Heterotopic ossification (HO) is a debilitating condition defined by the *de novo* formation of bone within non-osseous soft tissues, such as skeletal muscle. Despite continued efforts to identify the cellular and molecular events leading to HO, the mechanisms of pathogenesis remain elusive. This study utilises a novel *in vitro* model of skeletal muscle to better understand the pathology of HO. A muscle model was developed by seeding C2C12 myoblasts within a type-I collagen gel. Uniaxial tension was produced by anchoring the gel at opposing ends of a well plate using polyethylene flotation bars. The model was cultured with high glucose DMEM and 20% FBS for 4 days, after which the FBS was replaced with 2% horse serum to facilitate myoblast fusion along the axis of tension. After myotube formation the model was exposed to inflammatory factors both independently and in combination, and the results compared with untreated and 2D controls. Cells were released from the model by digestion with 0.1% collagenase, plated at $10x10^4$ cells/cm^2 and cultured in growth or osteogenic medium. The osteogenic potential of the cells was determined using Alizarin Red (AR) staining. The results obtained are novel and show that a population of cells released from the collagen constructs remained unfused and did not contribute to myogenesis. Culture of these unfused cells in osteogenic medium led to ossification, as determined by AR staining. No ossification was observed in 2D controls. Our data demonstrated for the first time that exposure to TGF-β1 and PDGF led to the formation of mineralised nodules, independent of osteogenic medium. This study identified the presence of an osteogenic/non-myogenic population of C2C12 myoblasts when cultured in a 3D collagen model. We also showed that exposure to inflammatory factors identified at the site of tissue damage can lead to ossification, and may contribute to HO.

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Disruption of *Wntless* Leads to Impaired Dentin Apposition and Short Molar Roots

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Wnt signaling plays an essential role in the tooth morphogenesis of the dental epithelium and mesenchyme. However, it remains unclear if Wnt ligands, produced from dental mesenchyme, are necessary for odontoblast differentiation and dentin formation. To address the role of Wnt signalling in odontoblast differentiation and dentin formation, we analysed odontoblast-specific *Wntless* (*Wls*), a chaperon protein that regulates Wnt sorting and secretion, conditional knockout mice. Odontoblast-specific disruption of *Wls* leads to severe defects in dentin formation and root elongation. Dentin thickness decreased apparently and pulp chambers enlarged in the mandibular molars of mutant mice. Although the initial odontoblast differentiation was normal in the mutant, odontoblasts became cuboidal and dentin thickness was reduced. In immunohistochemistry, Wnt10a, -catenin, type I collagen, and dentin sialoprotein were significantly down-regulated in the mutant odontoblasts. In addition, roots were short and root canals were widened. Cell proliferation was reduced in the developing root apex of mutant molars. Furthermore, Axin2 and Osx expression was remarkably decreased in mutant odontoblasts. Deletion of the *Wls* gene in odontoblasts inhibits odontoblast maturation and root elongation.

These results indicate that Wnt ligands produced in odontoblasts are required for dentin apposition and root elongation.
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Bone Metabolism of Ovariectomised (OVX) Ewes Chronically Treated with High Doses of Zoledronic Acid (ZOL): Experimental Model of Osteonecrosis of the Jaw Associated to Bisphosphonates (ONJBPs)

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Background: ONJ has emerged as a complication of bisphosphonate (BPs) treatment which induces a strong reduction in bone remodelling. Ewes could be a useful experimental model because, among others, they allow making oral cavity interventions. The objective was to evaluate the effect of high doses of ZOL (equivalent to cancer treatment) on bone remodeling and maxillary bone mass in OVX ewes.
Methods: Adult Corriedale ewes's were bilateral OVX or SHAM operated. Then, they received ZOL (n=6) or physiological solution (3 OVX and 3 SHAM). After one year (T=12) the first molar was extracted and a dental implant was done at T=24. A bone defect was done contra-laterally and calcium (Ca) and phosphorus (P) content was evaluated in the extracted material. After 4 additional months (T=28), ewes were sacrificed. Blood was drawn during all these interventions and serum Ca, P, crosslaps (CTX) and bone alkaline phosphatase (BAP) were evaluated. Hemimandible bone mineral content (BMC) was evaluated ex vivo.
Results: Results were expressed in the following order SHAM, OVX and ZOL: sCa (mg/dl): 9.1±0.8; 9.0±0.5; 8.9±0.4; sP (mg/dl): 4.63±0.21; 4.37±0.22; 3.96±0.36; BAP(mg/dl): 50.3±2.5; 58.3±0.6; 54.0±4.3; CTX; 644±8.7; 826±83*; 271±57*,**; Ca (g/g tissue): 0.707±0.263; 0.443±0.049*; 0.537±0.048*,**; P (g/g tissue): 0.290±0.084; 0.208±0.011; 0.211±0.048; BMC (g/cm2): 32.1±9.2; 24.0±6.1*; 42.3±3.0*,**. (* and **): $p<0.05$ vs. SHAM and OVX, respectively. OVX and SHAM survival was 100% and ZOL 77% (2 deaths). Two ewes showed ONJBPs for Actinomyces spp. No differences among the groups were observed in sCa and BAP levels; sP showed a tendency to be lower in ZOL, without differences between SHAM and OVX groups. At T=12, CTX increased in OVX and decreased in ZOL vs. SHAM group. ZOL treatment partially prevented the decrease in BMC observed in OVX group. At T=24, BMC was significantly lower in OVX and significantly higher in ZOL vs. SHAM ($p<0.01$).
Conclusion: Under our experimental conditions, two ewes treated with high doses of ZOL showed ONJBPs. These group also showed an increase in mandible BMC as a result of a great decrease in bone resorption. Dent. R. Davison doctoral thesis. Rio Negro National University. PICTO-2010-0181 and CONICET.
Disclosure: The authors declared no competing interests.

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Patterns Of Vertebral Ossifications In Spine Of Chronic Spinal Injury Patients

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Background: Chronic spinal cord injury has an important role in sensorimotor disorders which is associated with many complications and the rate of these complications should be certainly considered. The most important complications are the changes in bones and joints which come with pain and lead to increase in their motor disability. In this paper, the radiographic images were studied and the ossifications of spinal vertebra were described and also the type of changes and their prevalence was evaluated.
Methods: In this study, 500 patients and veterans with spinal cord injuries who were hospitalised in Khatam-Ol-Anbia Hospital in Tehran between 2011 and 2013 were examined. The spinal X-rays of the patients have been studied for spinal ossification, osteophyte, syndesmophyte, psoriasis spondylitis ossification, DISH ossification and the presence of shrapnel in the graph.
Results: Among the patients, 485 cases were males and 15 cases were females and the average age was 50 and the mean duration of injury was 26 years. 446 patients were paraplegic and 54 cases were quadriplegic. In paraplegic patients, osteophyte, syndesmophyte, psoriasis spondylitis ossification, DISH ossification and shrapnel, was 53.6, 6.7, 25.1, 5.4, 28.3 percent, respectively. In quadriplegic patients was 38.9, 1.9, 13, 5.6, 25.9 percent, respectively. There was a significant relationship between age and the number of involved vertebrae (P=0.000), psoriasis like ossifications (P=0.048) and large osteophytes (P=0.037), also between the duration of the injury and the number of involved vertebrae (P=0.008). In addition, the presence of shrapnel in the graph is correlated with large osteophytes.
Conclusion: Since the most frequent cause of chronic spinal injuries of our patients has been the injury by shrapnel, our results may not be extended to all patients with spinal cord injury. In lumbar spine radiography of the patients, osteophytes, the shrapnel and psoriasis like ossifications were mostly seen.
Disclosure: The authors declared no competing interests.

P421

Higher Sclerostin Levels in Patients with New Onset Diabetes After Liver Transplantation than in Patients with Normal Glucose Tolerance or Prediabetes

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Background: Sclerostin is an osteocyte-derived inhibitor of the Wnt/beta-catenin signalling pathway, which acts as a negative regulator of bone formation. Clinical studies have found increased serum sclerostin in patients with type 1 and

type 2 diabetes. But, there are none about the relationship between sclerostin and new onset diabetes after transplantation (NODAT). So, the aim of our study is to evaluate sclerostin patients with liver transplantation (LT).
Methods: Cross-sectional study in 85 LT patients. 75 g oral glucose tolerance test was performed (OGTT) and diagnostic ADA criteria were followed. Biochemical data: Plasma glucose (PG), (baseline, 60 and 120 min after OGTT). Serum sclerostin was measured in fasting samples (Enzyme immunoassay).
Results: 50 males, 35 females were included in the study. Mean age was 58.6±12.5 years. 6 patients (7.05%) showed diabetes (NODAT), 31 patients (36.4%) prediabetes and 48 patients (64%) normal glucose tolerance (NGT). Mean sclerostin was: 1.36±0.88 ng/ml in patients with NODAT, 0.81±0.26 ng/ml in patients with prediabetes, 0.78±0.38 ng/ml in the total group and 0.69±0.28 ng/ml in patients with NGT. Significant differences were found in sclerostin levels between NODAT and NGT patients ($p<0.001$) and NODAT and prediabetes patients ($p<0.01$). Significant correlations were found between sclerostin and fasting PG ($r=0.33$;$p=0.002$); 60-minute PG ($r=0.34$;$p=0.002$); 120-minute PG ($r=0.43$;$p<0.001$); age ($r=0.36$;$p=0.001$). Multiple regression analysis showed that only age and fasting PG remained as independent predictors of sclerostin levels (standardised beta 0.29, $p=0.006$; 0.24,$p<0.05$ respectively).
Conclusions: We have found that sclerostin was significantly correlated with plasma glucose and age. Moreover, these results showed that sclerostin levels were significantly higher after LT in patients with NODAT than in patients with prediabetes or NGT. We believe that large-scale studies are needed to investigate the link of higher levels of sclerostin in NODAT patients and increased fracture risk.
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P422

Skeletal Phenotype and Genotype Correlations in Adult Patients with Osteogenesis Imperfecta

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Osteogenesis imperfecta (OI) is a hereditary disease characterised by compromised connective tissue predominantly caused by mutations in the two collagen type I (COL-1) encoding genes, *COL1A1* and *COL1A2*. Symptoms, as fractures, bone deformity, hearing loss and blue sclera, reflect the ubiquity of COL-1 throughout the body. The study aim was to improve our understanding of the link between the molecular background and clinical manifestations by investigating the genotype, COL-1 structure, patient history, anthropometry and skeletal phenotype including DXA and HRpQCT in a large adult OI population. The study comprised 85 adult OI patients (Sillence: 58 OI type I, 12 OI type III, and 15 OI type IV). All patients underwent DXA, HRpQCT, biochemical screening and anthropometry. Medical history was collected. *COL1A1* and *COL1A2* were sequenced and 68 OI causing mutations identified in 66 families (46 in *COL1A1*, 22 in *COL1A2*). Analysis of COL-1 structure by SDS-PAGE was performed in a subset (n=67). The mutations located at the C-terminal end of both genes, were more severe and the mutations in *COL1A2* generally caused more severe OI. The patients with a qualitative collagen defect had lower lumbar spine aBMD ($p=0.004$) and more fractures ($p=0.002$) than patients with a quantitative COL-1 defect. They also had reduced height, 147±25cm vs. 161±10cm ($p=0.02$), sitting height, 72±12cm vs. 81±8cm ($p=0.003$), and armspan, 151±24cm vs. 169±13m ($p=0.002$, T-test). HRpQCT revealed significant differences between patients with OI type I and IV with more decreased vBMD ($p<0.005$), decreased cortical thickness ($p<0.001$) and reduced trabecular number ($p<0.005$) in the mildly affected OI type I patients compared with OI type IV. Since the most severe cases of OI have qualitative COL-1 defects, we suggest that extension of the routine patient evaluation with mutation screening and structural collagen analysis may provide valuable information in the long-term management of adult OI patients.
Disclosure: The authors declared no competing interests.

P423

Bone Turnover in Patients with Heterotopic Ossification after Spinal Cord Injury

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Background: The aim of our study was to determine the bone turnover markers in patients with spinal cord injury (SCI) with and without heterotopic ossification.
Methods: Markers of bone formation (Osteocalcin, serum type 1 procollagen (N-terminal) (tP1NP)) and bone resorption (serum collagen type 1 cross-linked C-telopeptide (β-CTx)) were determined by the electrochemiluminiscence immunoassay "ECLIA" for Elecsys user cobas immunoassay analyzer. In the study were included 23 patients with spinal cord injury – first group (average age 26.8 ± 3.9, duration of spinal cord injury from 3 to 12 months) and 23 healthy people's appropriate age and gender (average age 30.6 ± 6.0, years). In the first group included 11 patients with spinal cord injury with the presence of heterotopic ossification – subgroup I and 12 patients with spinal cord injury without heterotopic ossification – subgroup II.
Results: The results of examination showed that patients of first group had significantly higher bone markers than control group: P1NP (256.7±48.2 ng/ml vs 49.3±5.1 ng/ml, $p<0.001$), serum β-CTx (1.47±0.23 ng/ml vs 0.45±0.04 ng/ml, $p<0.0001$), osteocalcin (52.2±9.8 ng/ml vs 24.9±2.08 ng/ml, $p<0.001$). There were obtained that levels of bone remodeling markers in patients with HO were significantly higher in

comparison with patients without HO: P1NP (404.9±84.9 ng/ml vs 133.2±15.7 ng/ml, p<0.001), serum β-CTx (1.75±0.23 ng/ml vs 0.28±0.14 ng/ml, p<0.0001), osteocalcin (87.1±18.9 ng/ml vs 29.4±3.7 ng/ml, p<0.001).
Conclusion: The bone formation and bone resorption markers in patient of first group were significantly higher than in healthy individuals of appropriate age. The rate of bone turnover markers in patient with HO was considerably higher than in patient without HO and the process of formation dominated over the resorption in patient with HO.
Disclosure: The authors declared no competing interests.

P424
Low Serum 25-Hydroxyvitamin D Levels are Associated with Wnt Signalling Pathway in Cardiovascular Disease
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Vitamin D increases osteoblastogenesis, by stimulation of the signalling pathway Wnt / b-catenin, and represses expression of inhibitors of this pathway. Recent studies have suggested that vitamin D may be associated with glycaemic control, vascular calcification and cardiovascular disease (CVD). This study aims to evaluate the relationship between circulating 25-Hydroxyvitamin D (25-OHD), glycaemic control and inhibitors of the WNT pathway (sclerostin and DKK1) in patients with CVD. We conducted a cross-sectional study with 120 subjects (45 patients with CVD and 74 controls). The inclusion criteria for CVD were: ischaemic cerebrovascular disease, coronary heart disease and / or peripheral arterial disease. Surrogate markers of CVD (carotid intima media thickness [IMT], carotid plaques and aortic calcifications) were evaluated. Serum levels of DKK1 sclerostin and inhibitors were measured by ELISA immunoassay. Serum 25-OHD levels were significantly lower in CVD group (16.4 ± 8.1 ng / ml vs 20.7 ± 11.0 ng / ml, p = 0.025) patients with carotid plaque (14.0 ± 6.6 ng / ml vs 20.6 ± 10.9 ng / ml, p = 0.001) and vascular calcification (15.2 ± 6.6 ng / ml vs 20.1 ± 11.7 ng / ml, p = 0.012). Serum 25-OHD showed a negative correlation with fasting glucose (r = -0,254, p = 0.004), HbA1c (r = -0,186, p = 0.037), PTH (r = -0,205, p = 0.028), sclerostin (r = -0,191, p = 0.032) and IMT (r = -0,178, p = 0.037). The multivariate regression model shows sclerostin as the only variable independently associated with serum 25-OHD (β = -0201; CI = -0220 to 0.002, p = 0.047). Circulating 25-OHD could modulate Wnt signalling pathway by regulating the expression of sclerostin in patients with CVD. Supplementation with vitamin D could improve the health status in this population.
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P425
Does Chronic Hepatitis B Carrier Status Affect Bone Mineral Density Changes During Pregnancy?
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Background: Current literature has shown that non-cirrhotic chronic hepatitis can be associated with reduced bone mineral density (BMD). On the other hand, a significant fall in BMD has been observed during pregnancy as part of its physiological changes. The objective was to evaluate the association between BMD changes and chronic hepatitis B carrier status in low risk pregnant women.
Methods: Consecutive patients were recruited from a general obstetric clinic over a period of 9 months. Quantitative USG measurements of BMD were performed at the os calcis bilaterally between 14-20 weeks, and at 36-38 weeks. All women were routinely screened for chronic hepatitis B carrier status at booking and their hepatitis (HbsAg)carrier status was correlated with BMD changes.
Results: A total of 390 women were recruited, and the mean BMD loss from early to late pregnancy was 0.0301 g/cm^2, SD 0.043. There were 34 chronic hepatitis B antigen (HbsAg) carriers within this cohort (8.7%). There were no significant differences in the age, parity, height and early pregnancy body mass index between the HbsAg carriers and non-carriers. There were no significant differences in the early pregnancy mean BMD value in the HbsAg group as compared with the non-carrier group (0.602 Vs 0.591 g/cm^2)(p=0.56), nor in the mean BMD loss detected during pregnancy (0.035 Vs 0.029 g/cm$^{2)}$ (p=0.47).
Conclusion: Chronic hepatitis B antigen carrier status had no effect on the physiological fall in BMD during pregnancy.
Disclosure: The authors declared no competing interests.

P426
The Aetiology and Risk Factor Analysis in Hypercalcaemic Crisis
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Hypercalcaemic crisis is a rare but life-threatening condition involving the decompensation of hypercalcaemia (usually when serum calcium > 13-15 mg/dL) with significant disturbance to cardiac, renal, gastrointestinal and neurological function. Although major textbooks have thorough and detailed reviews of hypercalcaemia, there are no articles elaborating specifically the aetiology and pathophysiology of hypercalcaemic crisis. The goal of this study was to identify

the aetiologies and risk factors of hypercalcaemic crisis. We performed a retrospective review from 01/2012 to 05/2014 of patients with hypercalcaemia at our tertiary care centre and analszed their characteristics. Sixty-two patients with severe hypercalcaemia (adjusted serum calcium level by albumin ≥ 13.5 mg/dL) were identified from 262 of hypercalcaemia. Demographic and clinical characteristics were evaluated. Our study revealed that there were no differences in the aetiologies between hypercalcaemic crisis (pHPT/malignancy/others: 15%/60%/25%) and severe hypercalcaemia without crisis (pHPT/malignancy/others: 7.1%/64.3%/28.6%, P = 0.617). Compared with severe hypercalcaemia without crisis, the serum calcium level was significantly higher in hypercalcaemic crisis (16.9±1.8 mg/dL vs 14.8±1.1 mg/dL, P < 0.001). However, no differences in serum calcium level were observed among the subgroups of different aetiologies in hypercalcaemic crisis (P = 0.662) or severe hypercalcaemia without crisis (P = 0.423). The logistic regression analysis showed that serum calcium level (OR = 3.66; 95% CI: 1.83 to 7.31) and age (OR = 1.06; 95% CI: 1.00 to 1.13) were independent risk factors for hypercalcaemic crisis. The multivariate linear regression analysis showed that significant predictors of serum calcium level in hypercalcaemic crisis were age (β = -0.694, P = 0.001) and AKI (β = 0.449, P = 0.013). To our knowledge, this is the first and most comprehensive study to investigate the aetiology and risk factors of hypercalcaemic crisis. The accurate assessment of risk before investigating aetiology has an important place in hypercalcaemic crisis screening.
Disclosure: The authors declared no competing interests.

P427

Aromatase Inhibitors in Breast Cancer and Bone Status

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Background: The hormonomodulator character of breast cancer justifies the use of treatments that are the cause of hypogonadism, bone loss and an increased risk fracture. The objective of the study: Evaluation of bone status in patients followed for breast cancer, under anti-aromatase, admitted in rheumatology for bone status evaluation.
Methods: Prospective study, preliminary, conducted in 51 patients followed for breast cancer and views in rheumatology for evaluation of bone status. Inclusion criteria were patients followed for infiltrating ductal carcinoma, taking aromatase inhibitor, naive of anti-osteoporotic treatment. Exclusion criteria were patients taking osteoporosis treatment, those with other risk factors for bone fragility and those with a history of fracture. All patients had a complete physical examination. A calcium and phosphate levels, the dosage of vitamin D and parathyroid hormone was performed in our patients.
Results: Among patients, 38 met the inclusion criteria. The mean age was 59 +/- 10 years. All received radiation therapy and chemotherapy after mastectomy. All were postmenopausal. Breast cancer was unilateral in 36 cases. The average duration of taking hormone therapy was 3 years +/- 2. Mean serum calcium was 95 g / l, mean serum phosphorus at 34 g/l, the average value of vitamin D was 25 ng / ml. The average parathyroid hormone at 69.86 pg/l. 29 patients had a T-score <or equal to -2 to at least one site from the spine and hip. In contrast, 9 cases had a T-score> - 2 to 3 sites. Among the patients with T-score <-2, 16 cases had normal vitamin D status, and 13 were inadequate. These have benefited from vitamin D supplementation and then oral bisphosphonates.
Conclusion: Our results are conforming with those found in the literature. Treatments hormonomodulators of breast cancers cause bone loss and fracture risk, justifying osteoporotic patients before starting treatment. Bisphosphonate therapy is indicated for fractures and / or densitometric osteoporosis.
Disclosure: The authors declared no competing interests.

P428

A Benign Phosphaturic Mesechymal Tumour with Locally Aggressive Behaviour Overexpressing Growth Factor Receptors

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Tumour-induced osteomalacia is a rare paraneoplastic syndrome caused primarily by benign mesenchymal tumors. These tumours typically follow a benign clinical course and local recurrence occurs in less than 5%. We investigated a 49-year-old man with multiply-recurrent mesenchymal phosphaturic tumor. The patient presented with chronic muscle weakness, myalgia and cramps. His medical record included the diagnosis of oncogenic osteomalacia for which he was submitted to tumor resection in the left leg thrice before. Laboratory examination showed hypophosphataemia, hyperphosphaturia, and an elevated serum FGF23 level. Radical surgical approach (amputation to the level of the distal femur) was recommended due to the tumour-size and history of previous recurrences. Complete biochemical and clinical remission, however, was not achieved. Molecular analysis of the tumour cells demonstrated overexpression of growth factor receptors implicated in tumour angiogenesis (PDGFRA, PDGFRB and VEGFR) together with increased expression of FGF23 and PHEX. TIO is usually associated with benign phosphauturic tumours and when identified resection of the tumour leads to complete remission. The underlying pathophysiology of recurrences in these tumours is not known. This is the first report showing increased expression of growth factor receptors in a locally aggressive but histopathologically benign phosphaturic mesenchymal tumour.
Disclosure: The authors declared no competing interests.

P429

Osteoinsulosis Disseminata – a Benign Bone Condition of Disseminated Enostoses

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Introduction: Enostosis or bone island is a common incidental finding. These benign developmental lesions represent foci of cortical bone embedded within the trabecular network of cancellous bone. They are usually solitary and may be found in any bone but skull involvement is unusual.
Methods: We saw a 49-year old woman for a second opinion on disseminated spinal lesions on CT and MRI scans which were suspected to be osteoblastic metastases. She underwent surgery and radiation therapy for bilateral breast cancer in 2013 and took tamoxifen. She was in good general condition and had no axial complaints.
Results: CT scans showed numerous osteodense circular and ovoid lesions with speculated margins and normal surrounding bone in the pelvis, lumbar and thoracic vertebrae, sternum, scapula, humerus, ribs and clavicles. The lesions were hypointense on STIR and T1-weighted MRI images and were surrounded by normal signals. Cortices were intact. Bone scintigraphy, tumour markers and calcium metabolism were normal. Extensive imaging did not reveal extraskeletal metastases. A cervical and thoracic spine MRI, performed in 2009 for neck complaints showed the same lesions, unchanged to the recent and current images. X-rays taken in 2012 for joint complaints showed bone islands around the elbows and in the hands. The suspicion of osteoblastic metastatic disease was rejected.
Conclusion: We present a not earlier described skeletal condition, which we name osteoinsulosis disseminata. This benign condition consists of numerous bone islands diffusely spread throughout the skeleton. The single lesions have the typical imaging characteristics of enostosis. Osteoinsulosis disseminata differs from osteopoikilosis by the presence of numerous lesions in the axial skeleton and by the absence of longitudinal bone lesions. Bone scintigraphy is helpful to distinguish this benign condition from osteoblastic metastatic disease.
Disclosure: The authors declared no competing interests.

P430

Osteocalcin and Undercarboxylated Osteocalcin in Newly Diagnosed DM2 Patients Advised on Lifestyle Improvement

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Background: Insulin is the key link between energy and bone metabolism. In the presence of insulin osteoblasts secrete undercarboxylated osteocalcin, which promotes pancreatic beta-cell proliferation, insulin secretion and its tissue sensitivity, fatty tissue metabolism and energy expenditure thus contributing to euglycemia. The aim was to investigate osteocalcin and undercarboxylated osteocalcin in adult patients with newly diagnosed diabetes mellitus type 2 (DM2) advised on lifestyle changes for glycemic optimisation.
Methods: Study included 57 patients newly diagnosed with DM2 (21 F, 36 M; median, range; age 57 years, 30-80; BMI 29.2, 23.2-43.6; blood glucose 9.5 nmol/L, 7.9-15; HbA_{1c} 8.0%, 6.3-12.0). Patients were advised on life-style improvement, no blood glucose medication was prescribed, and re-evaluated after three months. Osteocalcin, undercarboxylated osteocalcin and crosslaps were measured by commercial kits, and other biochemical parameters by standard recommended methods.
Results: No difference for analysed data existed between sexes. At second visit (n=22) lifestyle changes were observed by statistically significant decrease of BMI, glucose, HbA_{1c} and indices of steady state beta-cell function. No difference for osteocalcin or undercarboxylated osteocalcin was found between visits. For the second visit (n=22) two pairs of parameters revealed statistically significant negative correlations between glucose and osteocalcin (p=0.01), and also glucose and crosslaps (p=0.046), considered unexpected.
Conclusions: Changes of osteocalcin and undercarboxylated osteocalcin with improvement of gylcemia were not found after three months, although optimization of HbA_{1c} goal (≤ 6.5%) was achieved solely by lifestyle advice. At the second visit interesting negative correlations of glucose with osteocalcin and also crosslaps as glycemic optimisation were observed. This might indicate the association of glucose and bone metabolism. Corresponding relationship of crosslaps and glucose reflects coupling of bone turnover actions, as both osteocalcin and crosslaps correlated with glucose in a positive fashion.
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P431

Osteoporosis and Primary Hyperthyroidism

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Background: The main consequence of bone hyperparathyroidism is the increase of bone remodelling. The X-Ray absorptiometry (DXA) has consistently found a decrease in bone density in cortical areas, particularly in the proximal third of the radius, to a lesser extent at the upper end of the femur. The lumbar spine is rarely achieved. The objective of our study is to determine the number of osteoporotic patients by DXA in primary hyperparathyroidism.
Methods: This is a single-centre study, analytical, conducted in Rheumatology, of 63 patients followed in consultation bone diseases for hyperparathyroidism. The inclusion criteria were the patients with primary hyperparathyroidism was confirmed. The exclusion criteria were patients with other risk factors of bone fragility (inflammatory arthritis, steroid medication, diabetes), apart from age and menopausal status. Bone mineral density was measured at three sites with the same device (Hologic), together with the phospho-calcic analysis before

parathyroidectomy. Osteoporosis was defined as classified by the World Health Organization.
Results: Among 63 patients, 30 had primary hyperparathyroidism, and 17 answered to the strict inclusion criteria. There were 15 women and 2 men. The mean age was 72 years. 13 patients were postmenopausal. The mean body mass index was 22. 12 cases had densitometric osteoporosis proximal 1/3 of the distal radius, associated with osteoporosis at the lumbar spine in 7 cases and femur in 6 cases. Osteoporosis was at three sites in 5 cases. Patients were treated with conservative measures or surgical treatment as the concensus NIH, 2009.
Conclusion: Our results are consistent with literature. The majority of patients had osteoporosis at the cortical site. However, other sites including the lumbar spine and femur may also be affected. it was revealed a predominant under PHPT with spinal disease group that represents about 15 to 20% and 59% of asymptomatic patients have a higher bone loss 10% to at least one of these sites.
Disclosure: The authors declared no competing interests.

P432

A Maxillo-Facial Operation in a Patient with Fibrodysplasia Ossificans Progressiva (FOP): are we Ready for it?

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Background: Fibrodysplasia Ossificans Progressive (FOP) is rare disease characterised by progressive heterotopic ossifications during life leading to extremely disabling immobility, thoracic insufficiency and early death. The underlying cause is a mutation in the activin A type I receptor gene. In FOP skeletal muscles and connective tissues are being replaced by heterotopic bone which may be induced by small traumas or not well understood flare-ups. The course is chronic but unpredictable. No medical options are available and operations strongly discouraged. We present the course of FOP in a Dutch patient aged 23 who insisted on oral facial operation after 13 years of progressive mandible-maxillar occlusion with progressive limitations of food intake and vomiting.
Methods: The clinical history was followed from diagnosis FOP in 2001 along with blood tests of infection and bone formation parameters. In 2001 and 2014 before the maxillo-facial operation bone scans (technetium-99m bound to oxidronaat), combined bone scan-single-photon emission computed tomographic and FDG-PET scans of the head were performed. After the operation on November 14th 2014 ^{18}F-fluoride PET scan and magnetic resonance imaging were performed. Classical FOP mutation was confirmed.
Results: In 2001, after a fall the patient developed increasing occlusion of the mandibular-maxilla with ongoing uptake of the bone scan tracer first at the left os zygoma and later at left maxilla with progressive bone volume locally. During the operation, ectopic bone fusing left zygoma to mandible and occlusive ectopic bone around the right coronoid processes were removed. Despite high dose of corticosteroids severe clinical as radiographical flare-up of both left and right masseter and temporalis muscles developed. Experimental therapy was started. The outcome will be known and presented during the ECTS.
Conclusion: Operation in FOP is still hazardous due to lack of therapeutically options. We showed that flare-ups can be measured by advanced combined scans.
Disclosure: The authors declared no competing interests.

P433

Serum Protein Levels, Bone Fractures and Nutrition in Patients with Rheumatic Diseases

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Background: The relationship between protein dietary intake, Bone Mass Index (BMI) and bone metabolism is controversial. A hyperproteic diet is linked to increased renal calcium excretion but there is no clear evidence of its relevance in the development of osteoporosis (OP). This study aims to evaluate the relationship between protein diet intake, BMI, parathyroid hormone (PTH) levels and fractures.
Methods: Clinical data collected included: fall occurrence; history of vertebral fractures; total serum protein, albumin, inorganic phosphate, calcium, parathyroid hormone, vitamin D and calcium urinary excretion levels and lumbar densitometry. Descriptive statistics, Mann-Whitney and Spearman correlation were applied for a significance of $p<0.05$.
Results: 276 subjects were included, 80,8% female, mean age 56,4 years. The most prevalent rheumatic diseases were: rheumatoid arthritis (RA), osteoarthritis (OA), Sjogren's syndrome, systemic lupus erythematosus (SLE). The average BMI was 27,1 kg/m^2, higher in patients diagnosed with OA. 32 patients had history of fractures. We found correlation between the occurrence of fractures and female gender, lower T-score at femoral neck and lower total serum protein levels. In subjects over 58 years, we found an association with body percentage and BMI, independent of muscle mass ($p<0,05$). We also found a relation between higher PTH and higher BMI.
Conclusions: In this population, fractures were commoner in women, with lower BMI and lower serum protein levels. Higher BMI and body fat percentage may be risk factors for fall occurrence in the elderly. Patients that had a lower BMI and reduced body fat content consumed more lean fish and showed a higher intake of milk. High PTH levels were correlated with higher BMI, which is in concordance with new evidence suggesting that overweight and obesity do not protect against OP.
Disclosure: The authors declared no competing interests.

P434

Characteristics of Vitamin D Deficient Saudi Women with or without Blunted PTH Response - a Cross-Sectional Study

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Background: Vitamin D deficiency is highly prevalent in Saudi women, however not all women show a rise in parathyroid hormone (PTH) in response to low vitamin D status. The aim of this study was to examine the differences in the characteristics of vitamin D deficient women with or without a blunted PTH response.
Methods: This was a cross-sectional study conducted in the "Center of Excellence for Osteoporosis Research" (CEOR). A total of 381, otherwise healthy women (aged 25-45 years), with vitamin D deficiency (serum 25(OH)D<50 nmol/ L) participated in this study. Women were divided into quartiles according to PTH levels and the highest and lowest quartiles were compared with regard to different anthropometric and biochemical factors.
Results: Women in the lowest PTH quartile (n=88) were significantly younger, had smaller waist circumference (WC), lower body mass index (BMI) and lower blood pressure compared with the highest PTH quartile. They also had higher total serum calcium, and phosphate levels. There were no significant differences in serum magnesium level or in kidney function.
Conclusions: These results suggest that a rise in PTH in response to vitamin D deficiency may be determined by extent of body fat, with a rise in PTH seen in vitamin D deficient women with higher BMI. Future studies to confirm these findings and to determine other factors affecting the vitamin D – PTH relationship in obese women who are at higher cardiovascular risk are indicated.
Disclosure: The authors declared no competing interests.

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Vitamin D Deficiency and Primary Hyperparathyroidism

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Background: Parathyroid hormone at high concentrations, increases the activity and the number of osteoclasts and bone resorption. Due to its osteoclast activity, it promotes excessive loss of bone mass. The prevalence of vitamin D deficiency appears to be important in the early hyperparathyroidism (PHP). There is little data on the prevalence of this deficiency in PPH cases, the severity of bone involvement, on the benefits and safety of vitamin substitution. The aim of our study was to assess the prevalence of vitamin D deficiency in patients followed for PHP.
Methods: This is an analytical study, single center cross of 63 patients followed in rheumatology, especially in consultation embrittling osteopathies for hyperparathyroidism. Were included patients in whom the diagnosis of primary hyperparathyroidism was confirmed by clinical and laboratory data, radiologic studies (cervical ultrasound, cervical Sista-MIBI scintigraphy) or after the results of the surgery. Were excluded patients followed for secondary hyperparathyroidism. The age and menopausal status were not determining factors. All patients benefited before treatment, a phospho-calcic analysis, 25(vitamin D) and parathyroid. The body mass index was calculated. Patients were thus classified into two groups, those with a normal value of vitamin D, and the second a vitamin D deficiency were compared at the end the mean value of the parathyroid in each group. Normal significance of the 25 (OH) vitamin D was retained> 26 ng/ml.
Results: Of the patients, 24 met the inclusion criteria. These were 22 women and two men. The mean age was 62 years. 18 women were postmenopausal. The mean body mass index was 22. The mean value of parathyroid hormone was 314,5 pg/ml. The mean value of serum calcium was 103.72 g/l. 11 patients had normal levels of vitamin D, and 13 were in deficit. The average value of parathyroid hormone was 282 pg/ml in the group with deficit versus 192.4 pg/ml in the group with normal vitamin D. In 11 cases, the treatment necessitated the surgery according to the NIH Consensus, 2009.
Conclusion: Our results are consistent with those of the literature. More than half of patients with hyperparathyroidism are deficient in vitamin D, the upper bound value of hyperparathyroidism and therefore the osteodensitometric bone loss. In PHP, the deficient of vitamin D is a risk factor of hypocalcaemia and "hungry bone syndrome" after surgery, and are increased in the absence of vitamin substitution. The carful vitamin D supplementation would decrease serum levels of parathyroid hormone and bone turnover. It would promote the recovery of the bone density. In addition, vitamin D is involved in other chronic diseases, and dosage is recommended in patients with osteoporosis or at risk for osteoporosis.
Disclosure: The authors declared no competing interests.

P436

Surgical Methods in Mouse Tooth Autotransplantation Research

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Autotransplantation of teeth is one of the possibilities to substitute the missing teeth in the dental arch. In comparison with prosthetic dentistry and implantology, autogenous transplantation is the most natural way of tooth replacement. Although success and advantages are prevailing, there are some complications like ankylosis or inflammation, which have to be solved. To understand regenerative processes after autotransplantations, several animal models and histological/molecular methods have been established. We review

recent achievements in autotransplantation techniques with using animal models and the evaluation of advantages and disadvantages of their using as showed also in our experiments. The most common laboratory animals used in autotransplantation research are mice and rats. Despite the monophyodont dentition and reduced dental formula together with hypsodont incisors and toothless diastema, numerous findings can be successfully extrapolated from rodents to humans. Various parameters of successful autotransplantation such as periodontal tissue regeneration, reinnervation or revascularisation have already been proved in the mouse. The autotransplantation of teeth currently has a firm place in the field of dentistry because of preserving the physiological properties of teeth, periodontal and alveolar bone.

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PAEDIATRIC BONE DISEASE

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Compound Heterozygosity for a Frameshift Mutation and an Upstream Deletion that Reduces Expression of SERPINH1 in Siblings with a Moderate Form of Osteogenesis Imperfecta

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Osteogenesis imperfecta (OI) is a heritable disorder characterised by bone fragility and increased fracture risk, ranging in severity from mild to perinatal lethal. Mutations in 17 genes have been implicated in this disorder; more than 90% of cases of OI are caused by heterozygous mutations in COL1A1 or COL1A2. Most of the remainder are recessively inherited and result from mutations in genes encoding proteins involved in the synthesis of type I collagen. SERPINH1 encodes the collagen chaperone HSP47 that binds to arginine rich sequences in the type I procollagen trimers and provides the final steps in the folding and stabilisation of the triple helical domain. Loss of both alleles in mice results in early embryonic lethality. Homozygous missense mutations in dachshunds and in one child resulted in a moderately severe form of OI with continuing fractures despite bisphosphonate treatment. In both instances, the identified missense mutations resulted in substitution of different interacting leucine residues by proline. We describe a family with non-consanguineous unaffected parents who have two children with moderate short stature, low bone density, fractures, and no evidence of dentinogenesis imperfecta. Both children are heterozygous for two mutations (allele 1; allele 2); one of each was derived from each parent. Initial study identified only a frameshift mutation in the last exon that did not lead to nonsense-mediated mRNA decay (c.1233dupT, p.Asp412*). Analysis of the expression of the two alleles in cultured cells indicated that the other allele was expressed, but was only about half as abundant as the frameshift-containing allele. High density CGH identified a 5.3kb deletion upstream from the translation start site that removed a region of DNase sensitivity identified in the ENCODE data set. This allele was inherited from the father, and the mRNA in his cells was also expressed at a low level, confirming that this domain has a regulatory function for SERPINH1. This class of mutation is rarely found in individuals with genetically determined disorders and would not be detected by whole exome sequence analysis. While whole genome sequence analysis could identify it, targeted gene studies may be the more efficient way of identification, especially in families such as this one, in which only one mutant allele is found, and appropriate cells are available to measure expression levels.

Disclosure: The authors declared no competing interests.

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Does Foetal Smoke Exposure Affect Childhood Bone Mass? The Generation R Study

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Background: Maternal smoking during pregnancy may adversely affect bone health in later life. By comparing the associations of maternal and paternal smoking and of prenatal and postnatal exposure with childhood bone measures we aimed to explore whether the suggested association could be explained by foetal programming or reflects confounding by familial factors.

Methods: In 5,565 mothers, fathers, and children participating in a population-based prospective cohort study, parental smoking habits during pregnancy and current household smoking habits were assessed by postal questionnaires. Total body bone mineral content (BMC), bone area (BA) and bone mineral density (BMD) were measured by dual-energy X-ray absorptiometry (DXA) at the median age of 6.0 (IQR 0.37) years.

Results: In confounder adjusted models, maternal smoking during pregnancy was associated with a 11.6 g (95% CI 5.6, 17.5) higher BMC, a 9.7 cm^2 (95% CI 3.0, 16.4) larger BA, a 6.7 g/cm^2 (95% CI 2.4, 11.0) higher BMD and a 5.4 g (95% CI 1.3, 9.6) higher BMC adjusted for BA of the child. Current weight turned out to mediate these associations. Among mothers who did not smoke, paternal smoking did not show evident associations with childhood bone measures. Also, household smoking practices during childhood were not associated with childhood bone measures.

Conclusions: Our results do not support the hypothesis of foetal smoke exposure affecting childhood bone mass via intrauterine mechanisms. Maternal smoking or related lifestyle factors may affect childhood weight gain rather than skeletal growth.

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Abnormally High and Heterogeneous Bone Matrix Mineralisation in Children After Kidney Transplantation

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Chronic kidney disease (CKD) in children is associated with multiple skeletal complications and growth impairment. Increased fracture incidence often persists after transplantation and could be linked to alterations in bone material properties. Bone matrix mineralisation density distribution (BMDD) based on quantitative backscattered electron microscopy (qBEI) reflects bone turnover (average tissue age) and mineralisation kinetics of the bone matrix. We compared BMDD in transiliac bone biopsies obtained from patients 8.0±6.4 years after kidney transplantation (n=9, age 17.0±1.9 years, height Z-score -1.8±0.8, GFR (mL/1.73m²): 42.89±10.13, with a healthy paediatric reference cohort (n=54) and children with CKD on dialysis (n=18). None of the subjects was treated with bisphosphonates or growth-hormone. Bone histomorphometry of transplant recipients revealed low to normal bone turnover (MS/BS Z-score -1.0±1.4). Osteoid indices were normal but mineralisation lag time was increased. qBEI showed an increase in the most frequent calcium concentration (+3.4%; +4.7%) and the portion of fully mineralised bone (3-fold; 11-fold) in trabecular and cortical compartments respectively, versus healthy children. The amount of lowly mineralised bone packets was elevated in cancellous bone leading to a marked heterogeneity of matrix mineralisation. None of the BMDD parameters in the transplant recipients were significantly distinct from non-transplanted children on dialysis. In conclusion, the abnormally high bone matrix mineralisation in transplanted children is similar to non-transplanted CKD patients, suggesting a history of low bone turnover with accumulation of fully mineralised bone packets. Even though at the time of the biopsy histomorphometry revealed only marginally decreased bone turnover, it seems probable that BMDD alterations acquired during the decline of kidney function are not completely corrected. The observed delayed osteoid mineralisation and the increased portion of lowly mineralised bone packets suggest a slowdown in the onset and accumulation of mineral in the newly formed osteoid possibly linked to CKD and concomitant glucocorticoid and immunosuppressive medication.

Disclosure: The authors declared no competing interests.

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Evaluation of the Main Data of Inflammatory Response and Content of the Nuclear Factor-kB to the Base Therapy of Juvenile Rheumatoid Arthritis

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The aim of our study was to estimate changes of the inflammatory response signs as well as content of NF-kB due to basic therapy of the JRA. At our study we had checked 68 children with JRA, among them we presented 32 boys and 36 girls that passed treatment in Vinnitsya regional clinical children's hospital. The average duration of the disease in the group of the children we examined was 18,2±1,3 months. Laboratory-instrumental methods included routine studies as well as estimation of the main signs of inflammatory response activity – inflammatory cytokines in serum (Interleukin-1β and Interleukin-6), content of the nuclear facor-kB while using ELISA method. Currency of the JRA in children characterised with high increasing of the inflammatory response signs especially C-reactive protein (6,55(4,2;9,8)) mg/l, IL-1β (7,3(3,5;11,9)) pg/l, IL-6 (6,8(4,5;10,6)) pg/l and NF-kB (6,76 (4,8; 9,1)) pg/l, that are in correlative connections with clinical signs (number of the injured and swelled joints, evaluation of the general condition of the child according to doctors and own response) of the disease activity (r_{xy} = +0,34 up to 0,62, $p < 0,01$). Due to the provided treatment between all examined children with JRA in 32 (47,0±5,1%) of the patients we got ACR 30 response, 23 (33,8±4,8%) achieved ACR 50 and in 4 (5,9±1,9%) estimated ACR 70 level. During the managing of the basic therapy in children with JRA we estimated decreasing of the IL-1β content in patients at the background of methotrexate administration (38,7±3,7%), at the second group with use of sulfasalazine (28,5±3,5%) and the third with leflunomide direction (29,1±5,1%), but significant decreasing of the IL-6 content, that is one of the main inflammatory mediators and as well NF-kB was found just in group of the patients with methotrexate administration (on 36,3±3,8% and 32,4±2,4% for NF-kB).

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A Systematic Review and Meta-Analysis of Calcium Intake in Early Life and Risk of Childhood Fractures

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Background: Environmental exposures, such as inadequate diet may be considered as risk factors of childhood fractures. When building optimal bone mass, the bone homeostasis is closely regulated by calcium absorption and reabsorption, making calcium essential for bone mineral accrual especially

during growth. A systematic review and meta-analysis was conducted to examine the association between calcium intake and childhood fractures.
Method: Studies published up until January 2014 were identified based on literature search in the MEDLINE, Web of Science and SCOPUS databases and hand searching in references by first author based on predefined inclusion criteria (study design, age 2-13 years, health status). Meta-analysis was done for case-control studies examining differences in mean calcium intake in the case compared with the control group. Random effect analysis was performed based on the effect-estimates derived as the differences in mean calcium intakes between the cases and controls. The corresponding standard errors were derived based on the standard formula related to differences in means through usage of the Ns and standard deviations.
Results: In total, twelve studies examined overall calcium intake, of which nine were case-control studies, one was performed with a case-cohort design and two were longitudinal studies. The literature identified in the systematic review, suggests that there may be a threshold effect for intakes of calcium below RDA or perhaps even a u-shaped association including the risk of fractures at both low and high intake levels, due to the contradictive results from studies examining mean calcium intake. The pooled effect size of the nine case-control studies, which had appropriate data for the meta-analysis showed no association (p=0.99) with fair heterogeneity (I^2=69.3%, p=0.001) using the random-effects model.
Conclusion: Dietary calcium intake was not consistently associated with reduced incidence of childhood fractures in neither the systematic review, nor the pooled results from the meta-analysis.
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Normal Reference Ranges for Serum and Urinary Osteocalcin in Healthy Finnish Children and Adolescents

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Background: Children and adolescents have high bone turnover marker (BTM) levels due to high skeletal growth velocity and rapid bone turnover. Paediatric normative values are vital for differentiating between normal and abnormal bone turnover, investigating skeletal diseases in children or monitoring responses to bone treatment. We aimed to establish sex- and age-specific BTM reference intervals in healthy children and adolescents and to assess the variation by anthropometric variables, pubertal status and fat percentage (fat%) on urinary (uOC), serum total (sOC) and carboxylated (cOC) osteocalcin values.
Methods: Clinical characteristics, serum and urinary samples, and fat% (DEXA) data from a cross-sectional cohort were available for a total of 175 healthy, 7-18 year old Finnish children and adolescents with normal bone mineral density (Z score between -2 and +2); 107 girls (mean age 13.8) and 68 boys (mean age 12.5). Two-site immunoassays were used to measure sOC, cOC and creatinine-adjusted uOC mid-molecular fragments.
Results: BTMs increased with puberty, correlated with pubertal growth which occurred and declined earlier in girls (after 11 years) vs. boys (14 years). The mean values and percentiles for total sOC, cOC, and uOC for sex-specific age categories and pubertal stages (pre-, mid- and post-puberty) were established. There was limited correlation between sOC and uOC, especially in younger boys, driven by age categories with progressively improving correlation with increasing age. The determinants for sOC, cOC and uOC varied; the uOC being most robust while age, height, weight, vitamin D status and PTH influenced sOC and cOC. Body fat% had no influence on sOC, cOC or uOC.
Conclusion: In young children (age <11) circulating OC reflects more growth status than bone metabolism. Thus its validity, similar to other BTMs, as a determinant of healthy bone status is limited.
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Ewing Sarcoma Inhibition by Disruption of EWSR1-FLI1 Transcriptional Activity and Reactivation of p53

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Ewing sarcoma is a small blue round cell tumour occurring in the bone and soft tissue of young children. The causative genetic defect underlying the development of this tumour is a translocation fusing an Ews gene to an Ets transcription factor family gene, most frequently resulting in the fusion genes Ews-Fli1 (~85% of all cases) or Ews-Erg (~10% of all cases). Despite knowledge of this driving molecular event, an effective therapeutic strategy is lacking. To test potential treatment regimes, we established a novel Ewing sarcoma zebrafish engraftment model allowing time-effective, dynamic quantification of Ewing sarcoma progression and tumour burden *in vivo*, applicable for screening of single and combined compounds. In Ewing sarcoma, the tumour-suppressor gene p53 is commonly found to be wild-type, thus providing an attractive

target for treatment. Here, we study p53-wild-type (EW7, CADO-ES1 and TC32) and p53-deleted (SK-N-MC) Ewing sarcoma cell lines to investigate the potentiating effect of p53 reactivation by Nutlin-3 on treatment with YK-4-279 to block transcriptional activity of EWSR1-FLI1 protein. Blocking EWSR1-FLI1 transcriptional activity reduced Ewing sarcoma tumour cell burden irrespective of p53 status. We show that simultaneous YK-4-279 treatment with Nutlin-3 to stabilise p53 resulted in an additive inhibition of p53-wild-type Ewing sarcoma cell burden, whilst not affecting p53-deleted Ewing sarcoma cells. Improved inhibition of proliferation and migration by combinatorial treatment was confirmed in vivo with zebrafish engraftments. Mechanistically, both compounds together additively induced apoptosis of tumour cells *in vivo* by engaging distinct pathways. We propose reactivation of the p53 pathway in combination with complementary targeted therapy by EWSR1-FLI1 transcriptional activity disruption as a valuable strategy against p53-wild-type Ewing sarcoma. In addition, a high-throughput engraft model in blastula-stage embryos is developed for the rapid screening of novel anticancer compounds. This HT-model proved to be suitable to screen the inhibitory effects of multiple compounds on various Ewing's sarcoma lines.

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Impaired Bone Health in Paediatric Survivors of Retinoblastoma After Early in Life Chemotherapy Treatment

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Background: Impairment of bone health in survivors of childhood cancer occurs frequently. Retinoblastoma (RB) is a malignant eye tumour developing in very young children. Treatment can include chemotherapy as an adjuvant or eye sparing treatment attempt. We conducted a cross sectional study to assess bone health in a paediatric cohort of survivors of who had received treatment with chemotherapy at an especially young age (mean age 0.76 ± 0.67 years).

Methods: The study (DRKS00003636) was approved by the local ethics committee. Thirty-eight survivors (14/38 female) were recruited at regular visits to the Children's Hospital Essen. Of these, 14 patients had unilateral, 23 bilateral and one patient trilateral RB. Datasets of 33 patients, who underwent polychemotherapy combined with local therapies, were available for statistical evaluation. Patients were evaluated at a mean age of 4.4 ± 3.9 (0.7 – 15.8) years. Polychemotherapy typically consisted of cyclophosphamide (4800 mg/m^2), etoposide (1800 mg/m^2), vincristine (9mg/m^2) and carboplatin (1200mg/m^2). Clinical and biochemical parameters of growth, pubertal development and bone health were obtained. The history of fractures and bone pain was assessed.

Results: The mean height SDS was -0.45 ± 1.8 (-2.92 – 1.21) and mean BMI SDS was 0.46 + 0.81 (-1.4 – 2.3). A 25 OH-vitamin D deficiency (VD: < 20 ng/ml) was common and observed in 52% of the patients. Almost 15% had developed secondary hyperparathyroidism, and abnormal readings for bone formation or resorption markers. Bone pain was reported by 7% of the patients and 9% experienced fractures of long bones after primary diagnosis. We found no difference in bone health between children with bilateral and unilateral disease, or between irradiated vs. non irradiated children.

Conclusion: In addition to a vitamin D deficiency, around 20 % of the survivors after early in life chemotherapy presented with bone pain and altered parameters of bone health. These are the children who might be at risk to develop serious bone health complications. Since identification of children at risk is difficult, we recommend long term monitoring and supplementation of vitamin D.

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STEROID HORMONES AND RECEPTORS

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P446

Serum 25(OH)D Deficiency in Young Adults is Associated with Low Dietary Vitamin D Intake and a Shorter 25(OH)D_3 Half-Life

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The elderly are a well-established group at risk of vitamin D deficiency. However, a high prevalence of vitamin D deficiency has also been reported in young healthy adults across Europe. Despite this, the causes of vitamin D deficiency in younger age groups have not been well characterised. The aims of this study were 1) to determine the relationship between age and circulating 25(OH)D in healthy participants in a UK population and 2) to determine the causes of any difference in circulating 25(OH)D with age. We conducted a cross-sectional study of 112 healthy younger and older adult men and women (ages 16 to 42 and 55 to 77). Anthropometric measurements, diet diaries, sunlight exposure questionnaires and blood samples were collected from all participants. 25(OH)D_3 half-life was measured using a low oral dose of a stable isotope of 25(OH)D_3. More of the younger age group had serum 25(OH)D below 30 nmol/l than

the older age group (67% vs 40%, *P*<0.01). Serum 25(OH)D (27.0nmol/l [SD 19.6] vs. 45.3nmol/l [SD 27.8], *P*<0.001) and measured free 25(OH)D (3.3ng/l [SD 1.54] vs. 4.37ng/l [SD 1.62], *P*<0.001) were lower in younger adults than in older adults. Dietary vitamin D intake was lower (2.64µg/d [SD 1.64] vs. 3.86µg/d [SD 3.03], P=0.04) and $25(OH)D_3$ half-life was shorter in younger adults (17.2 days [SD 3.44] vs. 18.7 days [SD 4.02], *P*=0.045) compared with older adults. Sunlight exposure did not differ between age groups. We conclude that there is a high prevalence of vitamin D deficiency in young healthy adults in the UK during the winter and this is likely due to lower dietary vitamin D intake and a shorter 25(OH)D half-life than in older adults.

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