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14–17 May 2016, Rome, Italy

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Abstract book

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ECTS 2016

ORAL POSTERS

POSTER PRESENTATIONS

Clinical Update

Management of osteoporosis with focus on osteoporosis in men, pregnancy and patients treated with glucocorticoids

CU1.1

Pregnancy associated Osteoporosis: Pathophysiology and management

Carola Zillikens
Erasmus MC, Rotterdam, The Netherlands.

Although the baby growing in its mother's womb needs calcium for skeletal development osteoporosis and fractures very rarely occur during pregnancy. Pregnancy- and lactation-associated osteoporosis with the occurrence of fragility fractures mainly of the vertebral bodies was first described as a syndrome Nordin *et al*. in 1955. It is most commonly observed in the third trimester or early post-partum in women presenting with severe and prolonged back pain and sometimes height loss. The prevalence is unknown and so far about 130 case reports have been reported. The etiology is not known but a role of calciotropic hormones, such as PTHrP has been suggested. In some cases low bone mass may have been present before pregnancy and investigation for underlying causes is necessary. There are no guidelines for treatment due to the lack of controlled trials. Another form of rare pregnancy-associated osteoporosis is called transient osteoporosis of pregnancy, also usually presenting in the third trimester of pregnancy with sometimes very severe pain while walking or standing usually localized in the hip, sometimes leading to hip fracture. Radiographs or MRI show severe localized loss of bone mass and edema. This condition usually fades within a few months after birth of the child. In this presentation we describe the clinical picture of women diagnosed with osteoporosis shortly after pregnancy. Potential causes of pregnancy-associated osteoporosis and clinical consequences will be discussed as well as issues to take into account concerning patient management.

DOI: 10.1530/boneabs.5.CU1.1

CU1.2

Male Osteoporosis: Secondary causes and evidence based treatment

Mattias Lorentzon

Abstract unavailable.

DOI: 10.1530/boneabs.5.CU1.2

CU1.3

Vertebroplasty and kyphoplasty; evidence based treatment of painful vertebral fractures?

Nicola Peel
Sheffield Teaching Hospitals, Sheffield, UK.

Vertebral fractures (VFX) are the most prevalent fracture associated with osteoporosis and often cause severe pain which can become chronic. VFX are associated with a high risk of further fracture, increased mortality, and reduced quality of life. Vertebroplasty and kyphoplasty are techniques involving the percutaneous injection of bone cement into one or more symptomatic fractured vertebrae. In vertebroplasty, cement is injected directly into the bone, while in kyphoplasty, cement is injected at low pressure into a cavity created by prior inflation of a balloon. The primary aim of both techniques is to relieve pain with an additional potential outcome from kyphoplasty of restoration of vertebral height. Early open studies suggested benefit from both procedures with most subjects reporting a significant early reduction in pain. More recent randomised controlled studies and meta-analyses of both procedures have cast doubt on the initial data with little evidence of pain control superior to that associated with sham procedures or standard pain management protocols. Complications are rare but severe adverse events are described including cement extravasation and embolization. It remains unclear whether these procedures may result in an increased risk of fracture in adjacent vertebrae.

Kyphoplasty but not vertebroplasty has been shown to increase vertebral height following VFX. Studies have demonstrated an association with reduced mortality which was stronger for kyphoplasty than vertebroplasty. The mechanism for this is unknown and may be coincidental but as a consequence, meta-analyses demonstrate cost-effectiveness and guidance including from the National Institute for Health and Care Excellence recommend use in clinical practice.

Evidence that kyphoplasty and vertebroplasty achieve the primary goal of symptom relief remains limited and the optimal selection of patients and timing of treatment remains unclear. Results of current RCTs may provide clarity and help establish their role in the clinical management of patient with painful osteoporotic VFX.

DOI: 10.1530/boneabs.5.CU1.3

CU1.4

Longterm management of Osteoporosis treatment: Treatment failure, treatment breaks

Richard Eastell

Abstract unavailable.

DOI: 10.1530/boneabs.5.CU1.4

CU1.5

Evidence versus emminence:Treatment of glucocorticoid induced osteoporosis in premenopausal women

Cyrus Cooper & Willem Lems
Amsterdam Rheumatology and Immunology Centre, Amsterdam, The Netherlands.

Oftenly, the question arises whether anti-osteoporotic drugs should be prescribed in glucocorticoid-treated premenopausal women. On the one hand, there are strong arguments to prescribe these drugs, when these patients are treated with high dose glucocorticoïds, and suffer from diseases that have a negative effect on bone, for instance SLE.

On the other hand, the absolute fracture risk is low, related to their young age, and finally, in women with childbearing potential, the possible negative effect of long-acting bisphosphonates should be discussed.

Prof Cyrus Cooper will present the evidence, and I will present shortly some background data and focus on some cases, and hope for an open and lively discussion!

DOI: 10.1530/boneabs.5.CU1.5

Management of rare bone diseases

CU2.1

Osteogenesis imperfecta in the adult: does age make any difference to the need of care?

Lena Lande Wekre
NKSD, Oslo, Norway.

Osteogenesis imperfecta (OI) is a genetic disorder of increased bone fragility and other connective tissue manifestations with a wide spectrum of clinical expressions. Even though the main problems come from the skeleton, we have to make an overall examination including skeletal deformities, joints and muscles, hearing, sight, teeth, heart, lungs and gastrointestinal organs to establish optimal follow up guidelines for people with OI. These examinations should then provide the basis for an individually adapted follow up where one has taken into account type of OI, age and specific findings.

There are several studies and reports on follow up routines and treatment of children with OI. Few studies have however described clinical and social aspects in adults with OI, and especially the consequences of ageing. Distinctions may be made between the biologic ageing process (age changes that all people share), and the "probabilistic ageing process" (age changes that may happen to some, but not all people as they grow older) which may be influenced by OI. This overlap of processes should also be taken into consideration when making follow up programs for adults.

The plan for supervision is meant to give information needed to prevent complications related to OI, and to limit the loss of function. It should be a tool for persons with OI (and their families), the GPs and other health-–professionals who are giving care to persons with OI. The presentation will give a systematic overview over key clinical alterations and suggestions for follow up routines.

Learning objectives

- Osteognesis imerfecta in adults is about much more than the skeleton
- Type of OI, age and clinical findings have to be taken in to account when making the plan for follow up

DOI: 10.1530/boneabs.5.CU2.1

CU2.2

Fibrous dysplasia in the adult

Roland chapurlat
Inserm umr 1033, Lyon, France.

Fibrous dysplasia of bone (FD) is a rare bone disease affecting one or several bones, due to a somatic mutation of GNAS responsible for abnormal differentiation of the osteoblastic cell lineage. The bone lesions may be associated with bone pain, fracture, deformity and neurologic compression. McCune-Albright syndrome is an association of FD, endocrine complications - mainly peripheral precocious puberty - and café-au-lait cutaneous spots.
The mutated cells produce a fibrous tissue within the trabecular compartment, but also an excess of RANKL and IL-6 leading to increased osteoclastogenesis and bone resorption. In polyostotic forms an excess in FGF23 can lead to renal phosphate wasting, which causes bone pain and fracture by osteomalacia. Two thirds of the patients are affected by a monostotic form. The diagnosis may include simple radiographs, computerized tomography (CT), magnetic resonance imaging (MRI) and histopathology combined with molecular analysis of GNAS. A bone scan has to be performed at least once to establish a map of all lesions. Specific forms need special attention because of the risk of complications. At the femur, the observation of a large lytic lesion may justify a preventive osteosynthesis. Cranio-facial lesions must be monitored for the risk of optic nerve compression, but also for growth hormone excess. The differential diagnosis with the meningioma is also of paramount importance at this site. The treatment of bone pain has relied on the use of bisphosphonates (mainly IV), which are efficacious in 80% of patients. More recently, denosumab has been entered into small clinical studies, with conflicting results so far. Tocilizumab - a monoclonal antibody to the receptor of IL-6 - is on trial to treat FD. Drugs targeting some specific mechanisms of FD bone pain may be developed in the future.

DOI: 10.1530/boneabs.5.CU2.2

CU2.3

Bone marrow oedema syndrome

Erik Fink Eriksen

Abstract unavailable.

DOI: 10.1530/boneabs.5.CU2.3

CU2.4

Emerging treatments of rare bone diseases – relevance for the adult patient?

Franz Jakob

Abstract unavailable.

DOI: 10.1530/boneabs.5.CU2.4

CU2.5

Paget's disease of bone

Stuart Ralston

Abstract unavailable.

DOI: 10.1530/boneabs.5.CU2.5

Allied Health Professionals Session

Speakers

AHP1.1

Bone Structure and Function: Organization & composition of bone, bone modelling and remodelling, bone cells

Tim Arnett
Department of Cell & Developmental Biology, University College London, London, UK.

It is not hard to gain a working understanding of the composition and function of bone that can offer useful insights into the causes of common bone disorders such as osteoporosis. Osteoblasts are the bone forming cells. They work in teams to lay down type 1 collagen fibres (similar to the collagen in skin and internal organs). The newly formed collagen in bone is initially soft and flexible. Osteoblasts possess the special ability to mineralise this collagen with a form of calcium phosphate (hydroxyapatite) in order to form bone, which is thus a composite material. The hydroxyapatite makes the bone hard and resistant to compression or bending, and the collagen holds it together and prevents brittleness. Osteocytes are osteoblasts that have become trapped in the bone matrix they are forming. Osteocytes are connected to each other (and to osteoblasts) by innumerable fine processes, forming a living network that comprises >90% of all the cells in bone. Osteocytes are thought to have a major function in detecting and co-ordinating the responses of bone to the slight deformation it experiences when subjected to shocks or loads. They may also play a key role in preventing bone from becoming too highly mineralised (and thus brittle). Osteoclasts are large cells that are responsible for the destruction of bone. They are formed from the fusion of immature white blood (or marrow) cells. Osteoclasts attach tightly to the surface of bone matrix and excavate characteristic, sharply-defined pits. This dramatic process, called bone resorption, involves dissolving away the calcium phosphate mineral and fragmenting the tough collagen fibres. Osteoblasts, osteoclasts and osteocytes work together throughout life to create, remodel and repair bone. In early life bone forms rapidly but is of relatively low quality and serves only a temporary role. Juvenile bone is eventually removed by osteoclasts and slowly replaced by high quality adult bone that is laid down in regular layers, rather like plywood. In adult humans, bone remodelling is highly responsive to alteration of mechanical loading but normally remains in overall balance. With advancing age, and in many disease settings, the destructive activity of osteoclasts can begin to outstrip bone formation by osteoblasts, leading to net bone loss. This process can be rapid in the "honeycomb" (trabecular) bone that is present inside the vertebrae and at the ends of the long bones. Bone resorption by osteoclasts is swift compared with the rate of bone formation by osteoblasts, and drugs that target osteoclasts have proven to be particularly useful for treating bone loss disorders.

DOI: 10.1530/boneabs.5.AHP1.1

AHP1.2

Pathophysiology of Bone Loss: Growth and loss, changes in microarchitecture, molecular mechanism, hormonal regulation, nutritional influence

Barbara Obermayer-Pietsch[1,2]
[1]Medical University, Graz, Austria; [2]CBmed, Graz, Austria.

Bone is a dynamic tissue as well as an endocrine organ, with a broad range of functions, such as static balance and locomotion, protection of internal organs, hematopoiesis, mineral storage and hormonal regulation. Bone growth and bone loss and functional changes during lifetime are important factors for human health.

Metabolic bone diseases may occur in many circumstances via a disturbed balance of the complex cellular interactions involved in bone microarchitecture and function. Osteoporosis and osteomalacia, chronic kidney disease with consecutive metabolic bone disease (CKD-MBD) or bone changes during primary hyperparathyroidism or diabetes underline the interaction of organ systems with bone in health and disease. Bone fractures and fracture healing are important topics to discover. We are going to understand more and more of the molecular mechanisms and the metabolic regulation behind these processes.

Thus, specific diagnostic and therapeutic tools have been established and more research is on the way. Therefore, the knowledge of systemic conditions and diseases, including the nutritional influences are important factors in the interpretation and prevention of detrimental influences on bone health and on human health in general.

DOI: 10.1530/boneabs.5.AHP1.2

AHP1.3

Biomechanical modelling of bone: predicting bone strength and fracture risk using engineering approaches

Mary Bouxsein
Washington, USA.

Abstract unavailable.

DOI: 10.1530/boneabs.5.AHP1.3

AHP1.4

Osteoporosis treatments: past, present and future

Nicola Napoli
Rome, Italy.

Abstract unavailable.

DOI: 10.1530/boneabs.5.AHP1.4

Abstract Presentations

AHP.OC1.1

Age-dependent changes in the bone marrow microenvironment

Anjali Kusumbe[1], Saravana Ramasamy[1], Tomer Itkin[2], Tsvee Lapidot[2] & Ralf Adams[1]
[1]Max Planck Institute for Molecular Biomedicine, Muenster, Germany; [2]Weizmann Institute of Science, Rehovot, Israel.

Blood vessels define the properties of local microenvironments in the skeletal system, play crucial roles in osteogenesis and provide niches for haematopoietic stem cells. The properties of niche-forming vessels and their changes in the ageing organism remain incompletely understood. We have previously identified a new capillary subtype in the murine skeletal system with distinct morphological, molecular and functional properties. These vessels are $CD31^{hi}/Emcn^{hi}$, localized to growth plate and endosteal region, 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. Here, we show that Notch signalling in endothelial cells leads to the expansion of haematopoietic stem cell niches in bone. While endothelial hypoxia-inducible factor signalling promotes neo-angiogenesis, it fails to induce arterialization and expansion of PDGFRβ-positive perivascular cells and thereby does not improve vascular niche function. In ageing mice, niche-forming vessels in the skeletal system are strongly reduced but can be restored by activation of Notch signalling in endothelial cells. These findings argue that vascular niches are part of complex, age-dependent microenvironments involving multiple cell populations and vessel subtypes.

DOI: 10.1530/boneabs.5.AHP.OC1.1

AHP.OC1.2

To measure or not to measure? Vitamin D and parathyroid hormone in patients with clinical risk factors for osteoporosis

Oliver Bock, Susanne Pyttel & Ute Dostmann
Promedio - Integrated Medicine, Leipzig, Germany.

Background

Despite the large amount of studies published on the association of vitamin D deficiency with higher incidence of falls and fractures, the threshold for a sufficient serum 25(OH)D concentration remains subject to a considerable debate. There has also been no clear consensus on the assessment and treatment of vitamin D deficiency.

Objective

To examine the prevalence of vitamin D deficiency and/or insufficiency and its impact on calcium/phosphate homeostasis as well as on bone turnover in a major German cohort of individuals with defined clinical risk factors (CRF) for

osteoporosis and fractures (acc.to German DVO Guideline 2009, and QFracture Score 2013).
Results
In 2014 we examined a total of 7,253 patients (mean age = 62.6 yrs (s.d. 13.9); f 64.4%, m 35.6%) with CRF for osteoporosis and fractures. The prevalence of 25(OH)D serum levels < 75 nmol/l was 87.7%. 25(OH)D serum levels below 50 nmol/l (deficiency) and 25 nmol/l (severe deficiency) have been detected in 55.0 and 15.7% of patients, respectively. Elevated PTH levels (> 65 ng/l) have been found in 20.9% of 5,119 samples tested - with an inverse correlation to 25(OH)D serum levels ($P < 0.05$) and positive relationship to increased bone turnover markers (B-AP, OC, DPD). The prevalence of secondary hyperparathyroidism (sHPT) was highest in patients with severe Vitamin D deficiency (35.3%) but common also in patients with 25(OH)D serum levels between 50 and 75 nmol/l (13.5%).
Conclusion
The high prevalence of vitamin D deficiency or insufficiency in a major cohort of patients with CRF for osteoporosis demonstrates the importance of routine measurements of 25(OH)D diagnostic and therapeutic purposes. The results put into question the approach adopted in various national guidelines which do not recommend 25(OH)D routine measurements. Additional consideration of PTH serum levels may contribute to a more adequate estimate of individual vitamin D supplementation needs.

DOI: 10.1530/boneabs.5.AHP.OC1.2

AHP.OC1.3

Treat-to-target in osteoporosis. Mith or reality? Results of a spanish Delphi study

Manuel Muñoz-Torres[1], Enrique Casado[2], Esteban Jódar[3], Xavier Nogués[4], Joan Miquel Nolla[5], Jose Manuel Quesada-Gómez[6], Laura Canals[7], Monica Balcells[7] & Luis Lizán[8]
[1]University Hospital San Cecilio, Granada, Spain; [2]University Hospital Parc Taulí, Sabadell, Spain; [3]University Hospital Quirón, Madrid, Spain; [4]Mar Institute of Medical Resarch (IMIM), Barcelona, Spain; [5]University Hospital Bellvitge, L'Hospitalet de Llobregat, Spain; [6]University Hospital Reina Sofía. IMIBIC. RETICEF, Cordoba, Spain; [7]Amgen, Barcelona, Spain; [8]Outcomes'10, University Jaume I, Castellon, Spain.

Objective
To define, by expert consensus, the criteria for the application of a Treat-to-Target (T2T) strategy in osteoporosis, in Spain, and to assess the adequacy of current treatments for it.
Material and methods
Six Spanish experts in osteoporosis formed the Scientific Committee that led the project and designed the questionnaire used in two Delphi rounds. The 24 items included in the questionnaire assessed the experts' wish (W) and prognosis (P) for each item to occur in 5-year time, in a seven-point Likert scale (1 = entirely disagree; 7 = entirely agree). Second round included items without consensus in the first. Consensus was defined as ≧75% of agreement (5–7) or disagreement (1–3) responses.
Results
The first round was completed by 112 out of 165 experts and the second by 106. A total of 59.8% of participants were rheumatologists with a mean of 21.3 years (s.d.:8.5) of clinical experience.
There was consensus on 70% of items. Consensus was established in the utility of T2T strategy to define therapeutic objectives, optimal follow-up and, therapeutic algorithm (W:96.4%; P:82.1%). Experts agreed on the utility of lack of fractures (W:99.1%; P:97.3%), bone mineral density (BMD) (W:91.1%; P:91.1%) and fracture risk reduction by FRAX (W:75.9%; P:84.0%) as therapeutic objectives. Treatment failure was defined as no BMD gain after 2 (W:81.3%; P:82.1) or 3 years (W:77.7%; P:75.9%), new fracture diagnosis within 2 (W:92.0%; P:92.0) or 3 years (W:90.2%; P:88.4%) or the absence of bone turnover markers (BTM) change after 6 months (W:75.0%; P:93.4%) or 1 year (W:90.6%; P:89.6%) of treatment. Except for strontium ranelate (W:76.4%; P:58.5%), consensus was reached for all available and upcoming novel therapies to achieve a therapeutic target trough T2T strategy application.
Conclusion
A T2T strategy in osteoporosis can be implemented in Spain, since therapeutic objectives, treatment failure and appropriate treatment choice for this strategy have been established.

DOI: 10.1530/boneabs.5.AHP.OC1.3

Muscle and Bone Session

Muscle & Bone

MB1.1

The bone and muscle connection – perspectives of a bone scientist
Michaela Kneissel

Abstract unavailable.

DOI: 10.1530/boneabs.5.MB1.1

MB1.2

Signalling pathways in muscle and bone – perspectives of a muscle scientist
Marco Sandri

Abstract unavailable.

DOI: 10.1530/boneabs.5.MB1.2

MB1.3

Mechnotransduction in muscle and bone
Astrid Bakker

Abstract unavailable.

DOI: 10.1530/boneabs.5.MB1.3

MB1.4

Principles of locomotion and adaptation throughout the lifespan
Michael Kjaer

Abstract unavailable.

DOI: 10.1530/boneabs.5.MB1.4

MB1.5

Sarcopenia and frailty – the clinical picture
Cornel Sieber

Abstract unavailable.

DOI: 10.1530/boneabs.5.MB1.5

MB1.6

Imaging bone and muscle – established and new techniques
Klaus Engelke

Abstract unavailable.

DOI: 10.1530/boneabs.5.MB1.6

MB1.7

Translational studies on muscle hypertrophy and function
Jesper Gromada
Regeneron Pharmaceuticals, Tarrytown, New York, USA.

Loss of skeletal muscle mass and function in humans is associated with significant morbidity and mortality. The role of myostatin as a key negative regulator of skeletal muscle mass and function has supported the concept that inactivation of myostatin could be a useful approach for treating muscle wasting diseases. The human monoclonal antibody REGN1033 is a specific and potent myostatin antagonist. Treatment of mice with REGN1033 increased muscle fiber size, muscle mass, and force production. REGN1033 was also tested in monkeys and healthy volunteers where it increased lean muscle mass to a similar extent, but less than in mice. However, short-term effects of REGN1033 on mass and function in the elderly with sarcopenia were modest. In this presentation, I will present our extensive muscle and serum profiling data with REGN1033 in mice, monkeys, and humans with the idea that this translational approach will predict human efficacy of novel agents prior to going into the clinic.

DOI: 10.1530/boneabs.5.MB1.7

MB1.8

Sarcopenia medications in clinical studies
David Glass

Abstract unavailable.

DOI: 10.1530/boneabs.5.MB1.8

MB1.9

Exercise regimens and musculoskeletal response
Jörn Rittweger

Abstract unavailable.

DOI: 10.1530/boneabs.5.MB1.9

MB1.10

Sarcopenia and nutrition
Ellen Freiberger

Abstract unavailable.

DOI: 10.1530/boneabs.5.MB1.10

Main Symposium

What is new – the year in review

S1.1

What is new: clinical bone research highlights
Nicky Peel

Abstract unavailable.

DOI: 10.1530/boneabs.5.S1.1

S1.2

What is new: bone basic science highlights
Christa Maes

Abstract unavailable.

DOI: 10.1530/boneabs.5.S1.2

Insights from Outside: New Frontiers in Therapy

S2.1

Targeted gene therapy
Angelo Lombardo

Abstract unavailable.

DOI: 10.1530/boneabs.5.S2.1

S2.2

Therapeutic potential of RNA interference
Achim Aigner
Rudolf-Boehm-Institute for Pharmacology and Toxicology, Clinical Pharmacology, University of Leipzig, Medical Faculty, Leipzig, Germany.

Since its discovery <20 years ago, RNA interference (RNAi) has proven to be a powerful tool for the downregulation of any target gene of choice. This also offers the use of RNAi-inducing small interfering RNAs (siRNAs) in a therapeutic setting, for the knockdown of pathologically overexpressed genes in various pathologies. Beyond siRNAs, this concept has been extended towards microRNAs (miRNAs) and their inhibitors.
While the target cell provides the RNAi machinery, the delivery of the siRNA is still the major bottleneck in therapeutic RNAi use. Non-viral strategies include, among others, chemical siRNA modification and its coupling to fusion partners for targeted delivery as well as various approaches based on nanoparticle formulation.
This presentation highlights the major issues in siRNA application and gives examples for solutions towards the goal of developing RNAi therapeutics. Beyond siRNA conjugates, the use of liposomal or polymeric nanoparticles for delivery is discussed. Preclinical and clinical examples are given.

DOI: 10.1530/boneabs.5.S2.2

S2.3

Genome editing
John van der Oost

Abstract unavailable.

DOI: 10.1530/boneabs.5.S2.3

Microbiome and musculoskeletal health

S3.1

Gut microbiota and bone metabolism
Claes Ohlsson & Klara Sjögren
Center for Bone and Arthritis Research, Institue of Medicine, the Sahlgrenska Academy, Gothenburg, Sweden.

The gut microbiota (GM), the commensal bacteria living in our intestine, performs numerous useful functions, including modulating host metabolism and immune status. Our recent studies demonstrate that the GM is also a regulator of bone mass and we propose that the effect of the GM on bone mass is mediated via effects on the immune system, which in turn regulates osteoclastogenesis. A role of the GM in bone metabolism is further supported by studies demonstrating that antibiotic, probiotic, and prebiotic treatments that impact GM composition regulate bone metabolism. Collectively, these studies suggest that the GM may be a novel therapeutic target for osteoporosis. Treatment with probiotics has already been shown to improve bone mass in rodent models of bone loss, but future randomized clinical trials are required to determine the possible effect of probiotics and other novel therapies modulating the GM composition on bone mass and fracture risk in patients with osteoporosis.
Access to cheaper sequencing and improved bioinformatics tools will allow metagenomic sequencing for the analysis of the GM composition in large prospective clinical cohort studies. This can be used to evaluate the predictive value of the GM composition as a biomarker for low bone mass and fracture risk. In addition, metatranscriptomics and metaproteomics will most likely be used to identify the microbial genes and proteins that have an impact on bone mass and fracture risk.
We propose a new cross-disciplinary GM–bone research field called 'osteomicrobiology', bridging the gaps between bone physiology, gastroenterology, immunology, and microbiology. Future studies are clearly warranted in this new research field to determine if the GM composition might be used as a biomarker for fracture risk prediction and to validate the GM as a possible novel therapeutic target for osteoporosis.

DOI: 10.1530/boneabs.5.S3.1

S3.2

Treatment perspectives for gut microbiota
Tim Spector

Abstract unavailable.

DOI: 10.1530/boneabs.5.S3.2

East Meets West

S4.1

Local and systemic drug delivery systems for bone tissue regeneration
Ling Qin

Abstract unavailable.

DOI: 10.1530/boneabs.5.S4.1

S4.2

Relevance of vitamin D to pathogenesis and treatment of osteoporosis
Toshio Matsumoto
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In order to maintain bone remodeling balance, it is important to keep enough Ca absorption from the intestine. Vitamin D is required to increase Ca absorption from the gut. Because vitamin D acts via activation to 1,25-dihydroxyvitamin D [$1,25(OH)_2D$] in the kidney, native vitamin D cannot exert its full effect when the activation process is disturbed. Renal impairment is the main cause of deterioration of the activation of vitamin D. Aging is associated with the development of chronic kidney disease, causing a reduction in the renal $1,25(OH)_2D$ production and intestinal Ca absorption. Such changes in the elderly

cause negative Ca balance, and play a significant role in the pathogenesis of osteoporosis. Therefore, osteoporosis treatment is almost always accompanied by vitamin D and Ca supplementation in the Western countries. However, considering the poor activation of vitamin D in the elderly, it is more plausible to supply with active vitamin D. In addition, Ca supplementation causes a rapid and transient rise in serum Ca, which is associated with an increase in vascular calcification and cardiovascular event. Especially, when Ca supplements are given with active vitamin D, excess amount of Ca is absorbed, causing hypercalcemia, hypercalciuria and renal impairment. Thus, we do not usually recommend Ca supplementation when active vitamin D is given to patients.
Among active vitamin D compounds, eldecalcitol increases bone mass and strength by reducing osteoclast formation and bone resorption with an increase in focal bone minimodeling in animals. A 3-year randomized, double-blind, clinical trial demonstrated that eldecalcitol reduces the incidence of vertebral and wrist fractures more strongly than alfacalcidol. The marked reduction in wrist fractures is suggestive of the effect of eldecalcitol in increasing muscle power and preventing falls. Further studies are needed to clarify its effect on physical function and falls.

DOI: 10.1530/boneabs.5.S4.2

S4.3

Development of osteoconductive and osteoinductive bone healing materials

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Autogenous bone graft has been used in the treatment of a fracture, non-union, and bone defect. However, bone graft substitutes have been developed and used because of the limited amount or donor site morbidity of the autogenous bone graft. Many kinds of bone graft substitutes including osteoconductive materials and osteoinductive materials are used for improving bone healing. Osteoconductive materials help to provide a three-dimensional structure to support the ingrowth of the capillaries, perivascular tissues, and osteoprogenitor cells from the host bone. Osteoconductive materials are calcium phosphate ceramics such as hydroxyapatite, tricalcium phosphate and bicomposite of both materials, calcium sulfate and bioactive glass-ceramics. Osteoinductive materials induce the osteoprogenitor cells to form new bone by supporting mitogenesis of the undifferentiated perivascular mesenchymal cells. The most common osteoinductive materials are recombinant bone morphogenetic proteins.
The aim of this lecture is to introduce osteoconductive materials such as artificial hydroxyapatite, tricalcium phosphate, $CaO\text{-}SiO_2\text{-}P_2O_5\text{-}B_2O_3$ glass-ceramics, whose development I have been involved in. Artificial hydroxyapatite and tricalcium phosphate have been used as bone graft extenders. $CaO\text{-}SiO_2\text{-}P_2O_5\text{-}B_2O_3$ glass-ceramics can be used as intervertebral disc spacers for the interbody fusion surgery. In addition, *E. coli*-derived rhBMP-2 was produced. *E. coli*-derived rhBMP-2 has the advantage of lower manufacturing costs than the existing Chinese hamster ovary cell-derived rhBMP-2 because of mass production. We proved the osteoinductivity of *E. coli*-derived rhBMP-2 using various animal models such as the rabbit posterolateral fusion model, the mini-pig anterior lumbar interbody fusion model and the mini-pig lumbar posterolateral fusion model. Recently, we performed a clinical trial in patients with degenerative lumbar spine. Finally, the osteoinductivity of AB204, which was created from chimeric ligands of rhBMP-2 and activin-A with segmental gene cloning methods, was confirmed. The osteoinductivity of AB204 was significantly higher than that of rhBMP-2.

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Molecular Clocks

S5.1

Circadian clocks, ageing and age-related diseases

Qing-Jun Meng

Abstract unavailable.

DOI: 10.1530/boneabs.5.S5.1

S5.2

Good times, bad times: (patho)physiology of diurnal rhythms

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Like most organisms, we have developed an internal time keeping system that drives daily rhythms in metabolism, physiology and behavior, and allows us to optimally anticipate to the momentum of the day. At the basis of circadian timekeeping lies an intracellular molecular oscillator in which a set of clock genes cyclically regulate their own expression with an approximate (circa) 24-hour (dies) periodicity. The mammalian circadian system consists of a light-entrainable master clock in the neurons of the suprachiasmatic nucleus (SCN) in the brain, and light-irresponsive peripheral clocks in the cells of virtually all other tissues. As the circadian clock drives rhythmic expression of up to 10% of the active genes (thereby conferring rhythmicity to a wide range of cellular processes such as, but certainly not limited to, energy metabolism, metabolic activation of drugs, detoxification, hormone synthesis, DNA repair and cell cycle control), it may not come as a surprise that disruption of the circadian system is associated with disease. Indeed, genetic disruption of the circadian system in rodent models by inactivation of clock genes has been found to increase tumor growth, accelerate aging, and disrupt metabolism. Moreover, our 24/7 economy requires many people to work at "non-standard" times. Recently, epidemiological studies have revealed a relation between disturbance of our body clock by repeated shift-work and an increased risk for developing pathologies such as cancer, metabolic syndrome and cardiovascular disease. This presentation will address the mechanism and biological/medical importance of the circadian clock and its impact on the etiology, treatment and prevention of disease, with special emphasis on the bone.

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Joint ECTS/ASBMR Symposium: Bone Therapeutics Update

S6.1

Treatment of X-linked hypophosphatemia with anti-FGF-23

Karl Insogna

Abstract unavailable.

DOI: 10.1530/boneabs.5.S6.1

S6.2

PTH and PTHrP treatment for osteoporosis

Ben Leder

Abstract unavailable.

DOI: 10.1530/boneabs.5.S6.2

Workshops – Clinical & Pre Clinical

Preclinical imaging beyond bone mass

WS1.1

L*iv*E imaging for *in vivo* cellular profiling

Ralph Müller
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The maintenance and adaptation of bone morphology results from orchestrated remodeling processes. These processes are locally coordinated by osteocytes with biochemical signals that result in increased or decreased bone formation or resorption activities. To better understand the morphology, we therefore have to understand how osteocytes determine dynamic morphometric parameters within their local microenvironment. Recently, a local *in vivo* environment (L*iv*E) imaging technique was developed using *in vivo* microCT in combination with histology and image processing. L*iv*E imaging allows to quantify the mechanical and remodeling *in vivo* microenvironment of hundreds of individual osteocytes for several weeks prior to histological processing. Here, we used L*iv*E imaging in trabecular mouse bone to show that dynamic morphometry is locally linked to quantitative single-cell gene expression. The 6th caudal vertebrae of adult female C57BL/6 mice ($n=9$) were imaged three times over a period of 2 weeks by *in vivo* microCT and 3D dynamic morphometry. Local strain energy density (SED) was calculated for each time point by micro-finite element analysis. Cryosections were registered into 3D microCT data. Osteocytes were identified, mapped into the microCT data, tracked back in time through L*iv*E imaging and grouped according to their SED values and remodeling state. By laser capture microdissection, osteocytes ($n=720$) were isolated in subpopulations containing ten osteocytes on average and their gene expression was analyzed. L*iv*E imaging showed that osteocytes around eroding surfaces blocked bone formation activities by Sfrp1 and increased local bone resorption by MMPs. Osteocytes in areas of high SED increased not only WNT signaling by expression of β-catenin and connexin43 but also increased the expression level of the extracellular matrix protein Col1a2 indicating perilacunar matrix remodeling. L*iv*E imaging allows bridging the gap between dynamic morphometry and biochemical signaling and thereby helps us to understand how osteocytes contribute to bone remodeling on the molecular level.

DOI: 10.1530/boneabs.5.WS1.1

WS1.2

Intravital microscopy of bone and bone marrow

Masanu Ishii

Abstract unavailable.

DOI: 10.1530/boneabs.5.WS1.2

WS1.3

Acoustic assessment of bone properties beyond bone mineral

Kay Raum

Abstract unavailable.

DOI: 10.1530/boneabs.5.WS1.3

Stem cells – can we go from bench to patient?

WS2.1

Skeletal stem cells

Pam Robey

Abstract Unavailable.

DOI: 10.1530/boneabs.5.WS2.1

WS2.2

The challenge of translating stem cell therapies to the clinic: what you need to show regulators

James McBlane

Abstract unavailable.

DOI: 10.1530/boneabs.5.WS2.2

WS2.3

Stem cell treatments for osteopetrosis

Anna Villa

Abstract unavailable.

DOI: 10.1530/boneabs.5.WS2.3

MicroRNA and bone

WS3.1

microRNA and WNT signaling

Eric Hesse
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Bone is a highly dynamic tissue that is constantly dismantled and rebuilt throughout life by the coordinated and balanced activities of matrix-resorbing osteoclasts, bone-forming osteoblasts and matrix-embedded osteocytes. This system is governed by interconnected signaling pathways, transcription factors, secreted regulators, genetic determinants and epigenetic cues including non-coding RNAs. Canonical Wnt signaling is among the most influential pathways regulating tissue development and homeostasis not limited to but including bone mass under physiological, pathological and therapeutic conditions. Recent evidence demonstrates that many activators and inhibitors of the canonical Wnt signaling pathway are regulated by microRNAs (miRNAs), small non-coding RNAs that are known to control several processes during tissue formation and maintenance, including cell lineage commitment and differentiation. MiRNAs regulate protein abundance by pairing to the 3' untranslated region of coding mRNAs, thereby repressing translation. Physiologically, this system contributes to balance pathway activities in response to signaling molecules. For instance in bone, miRNAs have been shown to participate in the control of osteoblast-mediated bone formation and osteoclast-related bone resorption, thereby contributing to bone mass maintenance. Under pathological conditions, an aberrant miRNA signaling network can promote the onset and progression of a disease such as various types of cancer and osteoporosis. In this context, miRNAs may function as disease-specific biomarkers. More importantly, miRNA delivery or antagonism has been reported to attenuate several diseases under experimental and pre-clinical conditions thereby emerging as novel therapeutic tools. This lecture will provide an update on the function of miRNAs in Wnt signaling in the context of bone health and disease. The important role of miRNAs as novel molecular regulators, diagnostic tools and therapeutic targets in musculoskeletal medicine will be emphasized.

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WS3.2

miRNA in diabetic bone disease

Ursula Heilmeier
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Epidemiological studies have found that patients with Type 2 Diabetes Mellitus (T2D) have a higher incidence of fragility fractures relative to non-diabetic individuals. Given the substantial morbidity, mortality and costs that emanate from T2D-related fractures, proper recognition of T2D individuals at increased fracture risk is indispensable. Although fracture risk in type 2 diabetics is routinely assessed with WHO-FRAX scores or DXA these methods show limitations. This highlights that other mechanisms, independent of BMD, might be driving the pathophysiology of diabetic bone disease. In this context,

microRNAs (miRNAs) are of special interest. MicroRNAs (miRNAs) are small non-coding RNAs that orchestrate gene expression on a post-transcriptional level and emerged as masterregulators of many cell processes including e.g. cell differentiation and senescence. They get secreted into the blood stream from cells of various tissues proportional to local disease severity where they remain stably expressed. Recent evidence has shown that miRNAs are crucial to bone homeostasis ("osteomiRs") and T2D etiology. This talk will focus on how studying miRNA expression may provide novel insights into the pathophysiology and morphological correlates of increased bone fragility in T2DM patients.

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WS3.3

Therapeutic implications
Speaker TBC

Abstract unavailable.

DOI: 10.1530/boneabs.5.WS3.3

Osteoporosis: Treat-to-target

WS4.1

Defining a goal for the treatment of osteoporosis
Cyrus Cooper[1,2]
[1]University of Southampton, Southampton, UK and [2]University of Oxford, Oxford, UK.

The fundamental purpose of osteoporosis treatment is to reduce the risk of fracture. There is no validated quantitative marker that monitors risk reduction in the individual patient; available treatments are effective, but reduce fracture incidence only by 20–60%, and it is to be expected, therefore, that fractures will arise during treatment. The aim of treat-to-target strategies is to simplify management and ultimately reduce organ damage and improve clinical outcomes. Such strategies have been widely used in cardiovascular and metabolic medicine; however, the role of a treat-to-target strategy in osteoporosis management remains an area for further research. The IOF and ESCEO have reviewed the most likely surrogate parameters currently available: BMD, BTM, FRAX and bone strength. None of these appears to be ready for use in a treat-to-target strategy. All the targets would be unattainable in many patients, even if targets could be agreed upon and validated. Additionally, applying a treat-to-target strategy in individual patients becomes problematic because of the small treatment-induced changes in the candidate parameters. The inability to extrapolate from statistically significant correlations in large clinical trials to make treatment decisions in individual patients, further limits the applicability of this concept in daily clinical practice. Research into the most effective goal-directed treatment of osteoporosis needs to be actively undertaken, and comprises the subject of working groups of the IOF.

DOI: 10.1530/boneabs.5.WS4.1

WS4.2

Which target to choose: a biomechanics perspective
Mary Bouxsein

Abstract unavailable.

DOI: 10.1530/boneabs.5.WS4.2

WS4.3

Uncoupling of resorption and formation; the tool to reach the treatment goal
Roland Baron

Abstract unavailable.

DOI: 10.1530/boneabs.5.WS4.3

Chronic kidney disease and bone

WS5.1

Bone fragility in chronic kidney disease
Sandro Mazzaferro

Abstract unavailable.

DOI: 10.1530/boneabs.5.WS5.1

WS5.2

Bone and mineral metabolism in end-stage kidney disease
Masafumi Fukagawa
Division of Nephrology, Endocrinology and Metabolism, Tokai University School of Medicine. Isehara, Japan.

Various abnormalities of mineral metabolism develop in patients with chronic kidney disease (CKD), which are further modified by therapeutic modalities including renal replacement therapy. Although secondary hyperparathyroidism is the most popular abnormality, it has been well recognized that uremic patients show skeletal resistance to PTH at the same time. Thus, bone metabolism in CKD patients shows very complex clinical presentations. Bone fracture occurs more frequently in CKD patients than in general population, however, such a difference cannot be explained sufficiently either by high PTH level or by decreased bone mineral density. We analyzed bones of CKD model rats, which showed abnormal mechanical properties despite comparable bone mineral density. We have shown by confocal Raman spectroscopy analysis that bone of these CKD rats contained more non-physiological collagen crosslink, abnormal crystallinity, and abnormal apatite orientation. Because these abnormalities were ameliorated by AST-120 treatment, uremic toxins may play important roles in deranged bone quality in CKD.

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WS5.3

Is PTH the good target to reduce fractures?
Pieter Evenepoel

Abstract unavailable.

DOI: 10.1530/boneabs.5.WS5.3

Update on Parathyroid Disease

WS6.1

The genetic bases of hypocalcaemia
Fadil Hannan

Abstract unavailable.

DOI: 10.1530/boneabs.5.WS6.1

WS6.2

Clinical consequences of long-term hypocalcaemia
Mike Mannstadt

Abstract unavailable.

DOI: 10.1530/boneabs.5.WS6.2

WS6.3

Management of hypoparathyroidism

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Recent studies have revealed hypoparathyroidism to be a disease associated with an increased risk of a number of co-morbidities including seizures, renal diseases, and infections as well as an impaired quality of life. In the summer of 2015, the first international guideline on treatment of hypoparathyroidism in the adults was published by the European Society of Endocrinology. The guideline is based on a systematic literature search for which available evidence was synthesized in order to assess: what is the best treatment for adult patients with chronic HypoPT? The Guideline suggest to treat patients with active vitamin D analogues (alfacalcidol or calcitriol) and calcium supplements in order to maintain serum calcium levels in the lower part or slightly below the lower limit of the reference range (target range) with patients being free of symptoms or signs of hypocalcaemia. Additional goals of treatment are to keep 24-h urinary calcium excretion within the sex-specific reference range, to avoid hyperphosphatemia, and to maintain the serum calcium–phosphate product below 4.4 $mmol^2/l^2$ (55 mg^2/dl^2). Patients should also have serum magnesium levels within the reference range and an adequate vitamin D status. The clinical importance of archiving these goals is, however, only poorly documented. Only sparse data exist on whether risk of complications is reduced in response to optimization of treatment. It is therefore of major importance that treatment is personalized with focus on the overall well-being and quality of life of patients. Aims to archive the biochemical targets should not impede the well-being of patients. In the future, replacement therapy with parathyroid hormone may become an alternative to conventional treatment as studies have suggested an improved QoL with a reduction in urinary calcium and serum phosphate level in response to PTH treatment.

DOI: 10.1530/boneabs.5.WS6.3

Hot Topic
Oral Communications

HT1

Storage disease and neurological phenotype in autosomal dominant osteopetrosis type 2 (ADO2). A preclinical study

Antonio Maurizi, Mattia Capulli, Juliana Cortes, Laura Di Rito, Nadia Rucci & Anna Teti
University of L'Aquila, L'Aquila, Italy.

ADO2 is a debilitating genetic bone disease causing multiple fractures and other severe symptoms. A mouse model of ADO2, harbouring the heterozygous $Clcn7^{G213R}$ gene mutation, phenocopies the human syndrome. The Clc7 gene encodes the ClC7 dimeric $2Cl^-/1H^+$ antiporter that is almost ubiquitously expressed, although the mutations hit especially the osteoclasts impairing bone resorption. By immunofluorescence, we observed that the mutant ClC7 was normally localized in the endoplasmic reticulum of monocytes and osteoclasts, significantly accumulated in the Golgi ($+30$-fold, $P=0.01$), which appeared enlarged and fragmented, and reduced in lysosomes (-0.52%, $P=0.02$). Lysosomes showed an impaired acidification (wildtype pH 3.63 ± 0.19; mutant 5.27 ± 0.68, $P<0.001$) improved by treatment with $Clcn7^{G213R}$-specific siRNA (pH 4.64 ± 0.69, $P=0.03$). The protein co-localised normally with OSTM1, suggesting no involvement of this pathway. In contrast, cells showed an increased expression of LC3, implying altered autophagy, typical of storage diseases. Since storage diseases may affect the brain, we subjected ADO2 mice to behavioural tests, observing increased anxiety (open field test, -48% time spent in centre; elevated plus maze test, -42% time spent in open arm; dark/light test, -46% time spent in lit compartment, $P<0.05$) and depression (forced swimming test, $+1.4$-fold time spent immobile, $P<0.01$). Conversely, memory, locomotion and aggressiveness were unaltered. Anxiety and depression worsened with age ($+1.7$, $P<0.05$), further supporting the hypothesis of a storage disease. Consistently, the Glo1 and Gad1 enzyme mRNAs, associated with anxiety/depression, but not the unrelated Gad2, Srr, Th and Dbh enzyme mRNAs, were increased in ADO2 brains (1.77 ± 0.5, 1.23 ± 0.07, respectively, $P<0.05$). Furthermore, β-amyloid aggregates accumulated in amygdala, cerebral cortex, hippocampus and thalamus, especially in aged (12 months old) ADO2 mice. Consistently, CLC7 protein accumulation was 15-fold increased in the Golgi of ADO2 primary neurons ($P<0.05$), which was 2.15-fold enlarged in hippocampus cryosections ($P<0.02$), confirming an aberrant localization of the mutant protein and the induction of a storage disease also in the neural cells.

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HT2

Mice lacking estrogen receptor α in hypothalamic POMC neurons display enhanced estrogenic response on cortical bone mass

Helen Farman[1], Sara Windahl[1], Deborah Clegg[2], Shang Kui Xie[2], Lars Westberg[3], Hanna Isaksson[4,5], Emil Egecioglu[3], Erik Schele[6], John Olov Johnsson[6], Juha Tuukkanen[7], Lisa Hahner[2], Jordan Zehr[2], Marie Lagerquist[1] & Claes Ohlsson[1]
[1]Medicine, Gothenburg, Sweden; [2]Internal Medicine, Dallas, Texas, USA; [3]Neuroscience and Physiology, Gothenburg, Sweden; [4]Biomedical Engineering, Lund, Sweden; [5]Clinical Sciences, Lund, Sweden; [6]Neuroscience and Physiology/Endocrinology, Gothenburg, Sweden; [7]Anatomy and Cell Biology, Oulu, Finland.

Estrogens are important regulators of bone mass and exert their physiological effects on bone mainly via estrogen receptor α (ERα). Central ERα has been reported to exert an inhibitory role on bone mass. ERα is widely distributed in the brain with a high expression in the arcuate nucleus (ARC) and the ventral medial nucleus (VMN) in the hypothalamus. Here, we tested the hypothesis that ERα in hypothalamic pro-opiomelanocortin (POMC) neurons, located in (ARC), is involved in the regulation of bone mass.

Six-month-old female POMC-$ER\alpha^{-/-}$ and control mice were ovariectomized (ovx) and treated with either vehicle or estradiol (E_2; 0.5 μg/day) for 6 weeks. As expected, E_2-treatment increased the cortical bone thickness in the diaphyseal region of femur, the cortical bone mechanical strength in tibia (Max load at failure) and the trabecular bone volume fraction (BV/TV) in both the distal metaphyseal region of femur and vertebrae L5 in ovx control mice. Importantly, for cortical bone thickness ($+126\pm34\%$, $P<0.001$) and mechanical strength (Max load at failure; $+193\pm38\%$, $P<0.001$), the estrogenic responses were substantially increased in ovx POMC $ER\alpha^{-/-}$ mice compared with the estrogenic responses in ovx control mice. In contrast, the estrogenic response on trabecular bone volume fraction was unchanged in the vertebrae L5 and only modestly augmented in the distal metaphyseal region of femur in POMC-$ER\alpha^{-/-}$ mice.

In a separate experiment, ERα in hypothalamic VMN was silenced using an adeno-associated viral vector, resulting in unchanged bone mass.

In conclusion, mice lacking ERα in POMC neurons display enhanced estrogenic response on cortical bone mass and mechanical strength. We propose that the balance between inhibitory effects of central ERα activity in hypothalamic POMC neurons and stimulatory peripheral ERα-mediated effects in bone determines cortical bone mass in female mice.

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HT3

Deletion of protease-activated receptor-2 improves bone and muscle pathology in dystrophin-deficient (mdx) mice

Neda Taghavi Esfandouni[1], Reza Sanaei[1], Chrishan Samuel[2], Charles Pagel[1] & Eleanor Mackie[1]
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Duchenne muscular dystrophy (DMD) is associated with osteoporosis, and dystrophic (dystrophin-deficient) mdx mice show reduced bone mass characterised by decreased mineral apposition and elevated bone resorption. To investigate a potential role of the G-protein-coupled receptor protease-activated receptor-2 (PAR_2) in the muscle and bone pathology associated with DMD, we established a colony of PAR_2-null-mdx mice. Limb and diaphragm muscles, tibiae and serum of male PAR_2-null-mdx and littermate mdx mice were examined every 4 weeks from just after the onset of muscle pathology (4 weeks) until 20 weeks of age. By 8 weeks, serum creatine kinase activity was lower in PAR_2-null-mdx mice compared to mdx, and continued to drop in PAR_2-null-mdx mice over time (70% lower than mdx, $P<0.0001$, at 20 weeks). From 8 weeks, in all muscles examined histologically, the area of active inflammation and the number of damaged fibres were lower in PAR_2-null-mdx mice compared to mdx mice. Hydroxyproline content in the diaphragm (indicative of fibrosis) was lower in PAR_2-null-mdx than in mdx mice from 12 weeks onwards (27% lower at 20 weeks, $P<0.01$). PAR_2-null-mdx mice showed significantly higher grip strength and lower fatiguability from 8 weeks of age (41% less fatigue, $P<0.001$, at 20 weeks). Micro-CT evaluation of the tibial metaphysis at 20 weeks of age showed that BV/TV, trabecular number and trabecular thickness were higher (by 80, 23 and 23%, respectively; all $P<0.01$) and trabecular separation was lower (14%, $P<0.05$) in PAR_2-null-mdx than in mdx mice. The serum concentrations of IL6 (88%, $P<0.01$), active TGFβ (19%, $P<0.01$) and RANKL (37%, $P<0.05$), and the RANKL/OPG ratio (49%, $P<0.01$) were lower in PAR_2-null-mdx mice compared to mdx mice at 20 weeks. These results suggest that PAR_2 activation contributes to muscle and bone pathology in dystrophin-deficient mice and that antagonising PAR_2 may help ameliorate the effects of dystrophin deficiency.

DOI: 10.1530/boneabs.5.HT3

HT4

Vitamin D supplementation in pregnancy leads to greater bone mass in UK infants born during winter months: the MAVIDOS multicentre, randomised, double-blind, placebo-controlled trial

Cyrus Cooper[1,2], Nicholas Harvey[1,2], Nicholas Bishop[4], Stephen Kennedy[5], Aris Papageorghiou[5], Inez Schoenmakers[6], Robert Fraser[7], Saurabh Gandhi[7], Stefania D'Angelo[1], Sarah Crozier[1], Rebecca Moon[1], Nigel Arden[3], Elaine Dennison[1], Keith Godfrey[1,2], Hazel Inskip[1,2], Ann Prentice[6], Zulf Mughal[8], Richard Eastell[9], David Reid[10] & Kassim Javaid[3]
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Maternal vitamin D status has been positively associated with infant bone mass in observational studies. We therefore evaluated whether 1000 IU/day cholecalciferol during pregnancy would lead to greater offspring bone mass at birth, in a UK, multicentre, randomised, double-blind, placebo-controlled trial (MAVIDOS, ISRCTN82927713).

At 12 weeks' gestation, pregnant women with a serum 25-hydroxyvitamin D [25(OH)D] 25-100 nmol/l were randomised to either 1000 IU cholecalciferol or matched placebo daily until delivery. Plasma 25(OH)D concentration was measured centrally at 14 and 34 weeks' gestation (Diasorin Liaison). Within 2 weeks after birth, infant whole body bone mineral content (BMC) was assessed by Dual-Energy X-ray Absorptiometry (Hologic Discovery, Hologic; or iDXA, GE-Lunar; measurements standardised).

Infants born to mothers supplemented with cholecalciferol had non-significantly greater whole body BMC than infants born to mothers taking placebo (total $n=665$; mean (S.D.) 61.6 (11.7)g vs 60.5 (11.1)g, respectively, $P=0.21$). However, in a pre-specified analysis, there was an interaction between season of birth and treatment allocation ($P=0.04$): infants born in winter (December–February) to mothers randomised to cholecalciferol had greater BMC than those randomised to placebo (63.0 ± 10.8 g vs 57.5 ± 10.9 g, $P=0.004$), a difference $>$ 0.5S.D. Similar patterns were observed for bone area and bone mineral density. At 34 weeks' gestation, the proportion of women with vitamin D sufficiency [25(OH)D $>$ 50 nmol/l] was increased (83.4% vs 36.5%, $P<0.001$) amongst those who received cholecalciferol compared to placebo. In the placebo group, 25(OH)D declined from 14 to 34 weeks' gestation in women who gave birth in winter or spring, but rose in those taking cholecalciferol, irrespective of birth season ($P<0.001$). No safety issues were identified.

Maternal gestational supplementation with 1000 IU cholecalciferol increases bone mass in UK infants born during winter months, and prevents the seasonal decline in 25(OH)D in these mothers. The findings inform public health policy relating to antenatal vitamin D supplementation. *CC and NCH are joint first author.

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HT5

Superior Gains in Bone Mineral Density (BMD) and Estimated Strength at the Hip for Romosozumab Compared With Teriparatide (TPTD) in Women With Postmenopausal Osteoporosis Transitioning From Bisphosphonate Therapy: Results of the Phase 3 Open-label STRUCTURE Study

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STRUCTURE was a phase 3, open-label study evaluating the effect of romosozumab or TPTD for 12 months in women with postmenopausal osteoporosis transitioning from bisphosphonate therapy (NCT01796301). This study enrolled women with postmenopausal osteoporosis who had taken an oral bisphosphonate for ≥ 3 years prior to screening and alendronate in the year prior to screening; had a BMD T-score ≤ -2.5 at the total hip (TH), lumbar spine (LS), or femoral neck (FN); and had a history of fracture. Subjects were randomized to receive subcutaneous romosozumab 210 mg QM or TPTD 20 μg QD. The primary endpoint was percent change from baseline in BMD by DXA at the TH through month 12. Secondary endpoints included percent change from baseline at months 6 and 12 in BMD by DXA at the TH, LS, and FN; hip integral and cortical BMD by quantitative computed tomography (QCT); and estimated hip strength by finite element analysis (FEA). The 436 women enrolled in the study had a mean age of 72 years and mean TH, LS, and FN T-scores of -2.2, -2.9, and -2.5, respectively. Through 12 months, the mean (95% CI) percent change from baseline in TH BMD by DXA was 2.6% (2.2, 3.0) with romosozumab and -0.6% (-1.0, -0.2) with TPTD ($P<0.0001$ between groups). Romosozumab also resulted in significantly larger BMD gains at the TH, LS, and FN at months 6 and 12 vs TPTD ($P<0.0001$). Significantly greater gains in integral and cortical hip BMD, and in estimated hip strength were observed with romosozumab vs TPTD at both time points (Table). The subject incidences of adverse events were generally balanced between treatment groups. In conclusion, in subjects transitioning from bisphosphonate therapy, romosozumab was well-tolerated and was associated with greater BMD gains and improved estimated hip strength compared with TPTD.

Table 1

	Cortical BMD by QCT		Integral BMD by QCT		FEA Estimated Strength	
	Month 6	Month 12	Month 6	Month 12	Month 6	Month 12
Romo	0.7	1.1	2.3	3.4	2.1	2.5
	(0.3, 1.1)*	(0.6, 1.6)*	(1.9, 2.7)*	(2.9, 3.8)*	(1.6, 2.5)*	(1.7, 3.2)*
TPTD	−2.7	−3.6	−0.8	−0.2	−1.0	−0.7
	(−3.1, −2.3)	(−4.1, −3.1)	(−1.1, −0.4)	(−0.7, 0.3)	(−1.5, −0.6)	(−1.5, 0.1)

Data are least squares means (95% CI). *$P<0.0001$ compared with TPTD.

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HT6

Effect of KRN23, a fully human anti-FGF23 monoclonal antibody, on rickets in children with X-linked hypophosphatemia (XLH): 40-week interim results from a randomized, open-label Phase 2 study

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In XLH, high circulating FGF23 causes hypophosphatemia, rickets, and short stature. In our Phase 2 study, 52 XLH children (ages 5-12 years, $\geq$Tanner 2) were randomized to receive KRN23 subcutaneously biweekly (Q2W) or monthly (Q4W). Serum phosphate (Pi) was measured biweekly. KRN23 dose was titrated (maximum 2 mg/kg) targeting age-appropriate serum Pi concentrations.

The first 36 subjects had a mean 6.6 years of standard-of-care treatment before washout. Serum Pi increased from baseline in all subjects to near normal levels (mean increase 0.30 mmol/l at 38 weeks; $P<0.001$) and was more stable with Q2W dosing; hyperphosphatemia did not occur. KRN23 significantly improved rickets, assessed by the Thacher Rickets Severity Score (RSS), with greater improvements seen with Q2W dosing (44% reduction; $P=0.0126$) and particularly in higher-severity rickets patients (baseline RSS ≥ 1.5) (59% reduction; $P<0.0001$). Using the Radiographic Global Impression of Change (RGI-C; -3 = worsening; $+3$ = complete healing), Q2W dosing improved rickets by $+1.6$ ($P<0.0001$) with the higher-severity rickets subset showing substantial healing ($+2.0$; $P<0.0001$). Alkaline phosphatase, a marker of rickets severity, decreased. Most treatment-related adverse events (AE) were mild, most commonly a transient injection site reaction (39%). One child experienced a serious AE and was hospitalized for fever/muscle pain that improved and continues in the trial. No clinically meaningful changes occurred in serum or urine calcium, serum iPTH, or renal ultrasound. In summary, KRN23 improved phosphorus homeostasis and rickets in children with XLH, with a favorable benefit-risk profile.

Table 1

		All Patients		Patients with baseline total RSS $\geq$1.5		
		Baseline	Wk 40		Baseline	Wk 40
Mean total RSS	All ($n=36$)	1.4	1.0*	All ($n=18$)	2.3	1.2*
	Q2W ($n=18$)	1.5	0.9*	Q2W ($n=9$)	2.4	1.0*
	Q4W ($n=18$)	1.3	1.1	Q4W ($n=9$)	2.2	1.4*
Mean RGI-C	All ($n=36$)		+1.4*	All ($n=18$)		+1.9*
	Q2W ($n=18$)		+1.6*	Q2W ($n=9$)		+2.0*
	Q4W ($n=18$)		+1.2*	Q4W ($n=9$)		+1.7*

*$P<0.05$, comparing Wk 40 to baseline.

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Cancer and Bone Oral Communications

Oral Communications

CABS.OC1.1

Secreted YB-1 (Y-box binding protein 1) as a biomarker of bone disease progression in patients with breast cancer and bone metastases

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YB-1 (Y-box binding protein 1) is a multifunctional cold-shock protein that has been implicated in all hallmarks of cancer. Elevated YB-1 protein levels were correlated with poor prognosis in several types of cancers, including breast cancer (BC). In BC, high YB-1 expression is a marker of decreased overall survival (OS) and distant metastasis-free survival across all subtypes. YB-1 is also secreted by different cell types and may act as an extracellular mitogen. Therefore, our aim was to evaluate the association between YB-1 serum levels and clinicopathological characteristics and clinical outcomes of patients with BC and bone metastases (BM). In this retrospective cohort study we included 44 patients diagnosed with BM from BC, which started therapy with bisphosphonates and had peripheral blood collected at the time of first treatment with bisphosphonates. YB-1 was quantified in serum using Human YBX1/YB1 Sandwich ELISA kit (LSBio), according to manufacturer's instructions. Clinicopathological characteristics were tabulated according to YB-1 status and differences tested using Fisher exact test. Time to event outcomes were analysed using Cox models. YB-1 was detected in the serum of 22 patients (50%), and correlated with the presence of extra-bone metastases ($P=0.044$), but not with other relevant clinicopathological characteristics. A non-significant trend towards estrogen receptor-negativity and radiographically mixed BM lesions was also found. Multivariate analysis showed that positive serum YB-1 is a marker of faster bone disease progression (HR 3.29, 95% CI 1.13–9.60, $P=0.029$). No significant differences were observed concerning OS (HR 2.04, 95% CI 0.86–4.87, $P=0.108$), and time to development of skeletal-related events (HR 1.45, 95% CI 0.53–4.00, $P=0.467$), although patients with positive YB-1 in serum had decreased median time to events.

Our data suggests that positive YB-1 in serum of patients with BC and BM is a biomarker of a more aggressive disease, with faster bone disease progression.

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CABS.OC1.2

Bone and metabolic parameters are associated with overall survival in patients with bone metastases from adenocarcinoma lung cancer: the POUMOS study

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Rational
Mortality due to non-small cell lung cancers is the first cause of cancer death in men around the world. Lung adenocarcinoma regularly induces bone metastases responsible for high morbidity and impaired life quality. Overall survival of these patients is poor. Thus we aimed to identify if some bone and metabolic parameters were associated with overall survival.
Patients and Methods
POUMOS is a prospective cohort of patients suffering from adenocarcinoma lung cancers with a first bone metastasis (stage IV). All patients had a bone biopsy with molecular status characterization of the tumor for EGFR, KRAS, BRAF and ALK. Bone metastasis localizations were obtained by bone scintigraphy or FDG-PET/CT. Whole body composition was obtained by DEXA scan (Hologic®): including bone mineral density and appendicular lean mass/height^2 (ALM index). We assessed fasting blood levels of glycated haemoglobin (HbA1C), calcemia, CTX and DKK1 (ELISA Teco®). Survival analyses were performed using a proportional hazard regression model.
Results
Between 2011 and 2014, we included 64 patients (75% men), aged 65 ± 11. Median survival was 30.5 weeks. There were 54 (84%) smokers, 39 (61%) had a good performance status (PS = 0/1). Liver and surrenal metastases were found in 19 (30%) and 20 (31%) patients. Molecular biology of the tumor revealed 9 (14%) EGFR and 11 (17%) KRAS mutations. Eighty percent of patients received combined chemotherapy. More than a half of patients had >5 bone lesions and 41% had a weight-bearing bone involvement. Smoking, PS >2, weight-bearing bone involvement, >5 localizations, hypercalcemia, elevated DKK1, elevated white cells, low ALM index and low HbA1C were significantly associated with poor overall survival.
Conclusion
In lung cancer patients with bone metastases, beside common prognostic factors, bone and metabolic parameters are independently associated with overall survival, suggesting that cares dedicated to bone metastases may be essential to improve prognosis.

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CABS.OC1.3

Blocking IL-1R signalling inhibits breast cancer growth and bone metastases by altering the tumour microenvironment

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Background
We have recently identified interleukin 1B (IL-1B) as a potential biomarker for predicting breast cancer patients at increased risk for developing bone metastasis. In mouse models, IL-1B and its receptor (IL-1R1) are upregulated in breast cancer cells that metastasise to bone compared with cells that do not. We have now investigated whether blocking IL-1R with the clinically licensed antagonist, anakinra, might be a potential treatment for breast cancer and bone metastasis.
Methodology
In vitro analysis of proliferation, migration and invasion were carried out in MDA-MB-231-IV, MCF7 and T47D cells treated with anakinra and/or IL-1B. For *in vivo* experiments mice received a subcutaneous implantation of MDA-MB-IV, MCF7 or T47D cells or intra-venous injection of MDA-MB-231-IV cells. Anakinra (1 mg/kg per day) or placebo was administered 3-days before (preventative) or 7-days later (treatment). Tumour volume was measured using callipers, apoptosis (TUNEL, Caspase 3), proliferation (Ki67) and angiogenesis (CD34, immunohistochemistry, VEGF and endothelin (qPCR)). Effects on bone were measured by uCT, and TRAP, P1NP, IL-1B, TNF alpha and IL-6 ELISA.
Results
Anakinra significantly reduced growth of MDA-MB-231-IV tumours in bone from $6.50\pm3.00\,mm^2$ (placebo) to $2.56\pm1.07\,mm^2$ (treatment) and $0.63\pm0.18\,mm^2$ (preventative). Anakinra also reduced the number of mice that developed bone metastasis from 90% (placebo) to 40% (treatment) and 10% (preventative). Anti-tumour effects were not confined to bone, subcutaneous tumour volumes reduced from $656.68\,mm^3$ (placebo) to $160.47\,mm^3$ (treatment) and $31.08\,mm^3$ (preventative). Anakinra did not increase tumour cell apoptosis but reduced proliferation and angiogenesis in addition to exerting significant effects on the tumour environment reducing bone turnover markers, IL-1B and TNF alpha. Anakinra had no effect on proliferation, migration or invasion of breast cancer cells *in vitro.*
Conclusions
Anakinra inhibits breast cancer growth and bone metastases *in vivo* by altering the tumour microenvironment.

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CABS.OC2.1

Prostate cancer microRNAs in extracellular vesicles stimulate osteoclastogenesis

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Prostate cancer (PCa) is the most common cancer in males. When patients develop metastasis, no curative therapy is available. To find new therapeutic options, it is crucial to understand how PCa cells induce metastasis. Recently, it was shown that PCa cells secrete small extracellular vesicles (EVs) that can be found in the circulation and in bones. Uptake of EVs by other cells may change their behaviour. We previously identified three miRNAs that were uniquely upregulated in metastatic PCa patients, though their contribution to metastasis remains to be studied. Therefore, we transduced PC-3, DU145 and RWPE-1 cells with three microRNAs resulting in overexpression. Effects on osteoclasts were determined by osteoclastogenesis and activity assays (coomassie staining of resorption pits) on bone chips using isolated human peripheral blood mononucleated cells. miRNA expression was determined by qRT-PCR, and PCa cell malignancy by the transwell invasion assay. PCa-cell invasion was stimulated by overexpression of two of the three microRNAs. Interestingly, in prostatic non-cancer RWPE-1-cells, a higher expression of the third microRNA increased the invasive potential, indicating that microRNAs have different functions at different progression stages. Importantly, the cellular miRNA levels correlated to the EV-miRNA levels. To determine whether the content of exosomes is actively used by osteoclasts, we used a Cre-lox system. THP1-monocytes were transduced with a reporter-construct, and two PCa cell lines with Cre, leading to active secretion of Cre within the PCa-EVs. When the EV-content is functionally used by the recipient THP1-cells, Cre recombines the reporter-gene resulting in a colour-switch. Indeed we observed a functional uptake in $>50\%$ of THP1-reporter^{+} cells, indicating that EVs can affect osteoclasts. Furthermore, osteoclastogenesis was increased when PCa-EVs were added. In conclusion, we identified miRNAs that enhance the malignant behavior of PCa-cells, and may also prepare the bone metastatic niche by activating osteoclasts at a distance through EVs.

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CABS.OC2.2

Integrin α5 is an independent prognosis factor and a potential therapeutic target for breast cancer bone metastasis

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Aims
Using an extensive bioinformatic approach we identified integrin α5 subunit as a novel potential target to treat bone dissemination from breast cancer. Aim of this study is to confirm the value of this target.
Methods
Integrin α5 mRNA expression levels were quantified by qRT-PCR, using radically resected primary tumors of 427 breast cancer patients. α5 expression at protein level by IHC on primary tumor was correlated, in an additional cohort consisting of 268 radically resected breast cancer patients, with presence of disseminated tumor cells (DTCs) in bone marrow at the time of surgery. BALB/c mice were injected in to tail vein with human MDA-MB-231 α5 silenced or scramble. Animals were killed on day 14 after tumor cell inoculation and lung and bone marrow from the hind limbs was flushed for DTC colony assay. Alternatively female BALB/c mice were treated with a chimeric monoclonal antibody that specifically binds to human integrin subunit α5 (M200) or with vehicle. Treatment was performed three times per week starting the day before intra-arterial inoculation with luciferase expressing human MDA-MB-231/B02 breast cancer cells, which selectively metastasize to bone. Vehicle and M200-treated mice were analyzed by radiography and bioluminescence. In an additional protocol, animals were culled on day 7 after tumor cell inoculation, and the bone marrow was flushed for DTC colony assay.
Results
Compared to low expression, an high α5 expression was associated with shorter bone metastasis-free survival, both in univariate ($P=0.024$) and multivariate analysis ($P=0.04$). Moreover we found significant ($P=0.039$) positive association between α5 protein expression on primary cancer and presence of DTCs in bone marrow. Taking advantage of the ability of the MDA-MB-231 cancer cell to metastatize both in bone and lung, we found that abrogating α5 gene function dramatically reduced the number of bone micrometastases ($P=0.015$) without affecting lung dissemination. In addition treatment of MDA-MB-231/B02 injected animals with M200 antibody significantly delayed the onset of skeletal lesions ($P=0.02$) and caused a 50% reduction in the extent of osteolytic lesions ($P=0.038$), compared to vehicle. This difference was accompanied with a sharp reduction of tumor burden ($P=0.02$), as determined by bioluminescence. Histomorphometric analysis of metastatic legs showed that M200 treatment decreased skeletal tumor burden ($P=0.027$) and increased the bone volume ($P=0.02$), compared to vehicle. Additionally, the number of DTC colonies from M200-treated mice was dramatically decreased compared with vehicle ($P<0.001$).
Conclusion
Our results suggest that α5 integrin expression in breast cancer cells facilitates bone marrow micrometastasis formation and the subsequent development of osteolytic lesions. vehicle ($P<0.001$). colonies in the bone marrow from M200-treated mice was dramatically decreased compared with vehicle ($P<0.001$).

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CABS.OC2.3

Cancer cell homing to the bone marrow is modulated by the mesenchymal stromal cell

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Several cell types form the hematopoietic stem cell niche. These niches sometimes become hijacked by cancer cells, which may later form metastatic lesions. Our aim was therefore to characterize the bone marrow microenvironment to affect cancer cell homing to bone marrow.
Pharmacologic modification consisting of PTH to stimulate the osteoblasts and zoledronic acid to prevent the increase in osteoclasts through PTH resulted in increased homing of cancer cells to the bone marrow 24 h after intracardiac injection of MDA-MB-231 cancer cells. Hematopoietic stem and progenitor cells numbers remained unchanged. Instead, we found a negative correlation between cancer cell homing and the decrease in mesenchymal stromal cells (MSC) ($r=-0.59$, $P<0.001$, $n=40$). Evaluation of a conditional knockout of β1 integrin in the bone marrow confirmed an association between diminished homing and increased MSCs. We next labeled and introduced MSCs into immune-deficient mice to determine the effect of increasing MSCs in the bone marrow. The labeled cells in the bone marrow correlated with the numbers injected. In addition, the more cells were detected in the bone marrow the less cancer cells homed to the bone marrow.
We then asked whether MSCs could be used as a prognostic marker for bone metastasis. In a cohort of prostate cancer patients, we found a correlation between the percentage of MSCs and the presence of epithelial cells in the bone marrow ($r=-0.47$, $P<0.005$, $n=35$). In addition, the percentage of MSCs by flow cytometry was threefold lower in patients with epithelial cell in the bone marrow than those without such cells.
These data suggest that either a lower number of MSCs is associated with more "space" for cancer cells to settle in the bone marrow, or that MSCs inhibit cancer cell homing. It is tempting to conclude that modification of MSCs might prevent cancer cells from homing to bone marrow.

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CABS.OC2.4

Peripheral tumour re-growth following combination therapy – role of the bone microenvironment

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Background
Cancer patients often receive a combination of drugs that target both the microenvironment and the tumour cells. However, the role of the bone microenvironment (BME) in mediating peripheral breast cancer growth remains poorly understood. This is the first study to determine whether reduced subcutaneous tumour growth following combination therapy is due to direct interactions between the drugs and tumour cells or through zoledronic acid induced alterations to the BME.

Methods
5×10^5 MDA-G8 cells were subcutaneously inoculated into female BALB/c nude mice. Animals received combination therapy (2 mg/kg doxorubicin (DOX, i.v.) followed 24 h later by 100 μg/kg zoledronic acid (ZOL, i.p.)) or PBS control weekly for 5 weeks. Tumour fragments (~14 mm^3, from mice receiving combination therapy) were subcutaneously transplanted into naïve BALB/c nude mice that had been pre-treated with 100 μg/kg ZOL or control. Tumour growth was monitored using callipers and alterations to the BME assessed (μCT, immunofluorescence, histology). Effects on bone marrow derived cells were determined by flow cytometry using markers for CD11b/Gr1+ and LSK cells.
Results
Weekly combination therapy successfully suppressed tumour growth (Tumour volume day 33: DOX/ZOL: 82.9±13.9 mm^3, $n=13$ vs. PBS: 330.9±125.3 mm^3, $n=5$, $P\leq0.0001$). Trabecular bone volume increased after both combination therapy (DOX/ZOL: 15.53±0.91% vs. PBS: 10.25±0.36%, $n=3$/group) and ZOL pre-treatment (ZOL: 37.04±1.80%, $n=4$ vs. PBS: 9.59±0.40%, $n=6$, $P\leq0.01$). Growth of re-transplanted tumour fragments resumed at equal rates whether transplanted into ZOL or PBS pre-treated hosts (Tumour volume day 33: ZOL: 223.9±45.36 mm^3, $n=8$ vs. PBS: 175.5±44.39 mm^3, $n=7$). Alterations in tumour and bone vasculature were observed following both ZOL or combination therapy.
Conclusion
Here we demonstrate that modification of the BME with ZOL is not sufficient to supress peripheral breast cancer growth following cessation of combination therapy suggesting that both the tumour and microenvironment need to be targeted for successful anti-cancer therapy.
Experiments performed under UK Home Office Authority (PPL40/3531).

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CABS.OC2.5

Inhibition of BMP signalling reduces bone destruction and impacts niche maintenance in a mouse model of multiple myeloma

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Multiple myeloma is usually incurable, the bone marrow niche providing a protective microenvironment for quiescent tumour cells. We hypothesised that manipulation of BMP activity, a regulator of cell differentiation in the bone marrow, might affect control of tumour growth by its niche and in addition alter the lytic bone disease of myeloma. Moreover BMP signalling contributes to the hepcidin upregulation and resultant inflammatory anaemia that is characteristic of myeloma. Regulation of BMP signalling within the myeloma bone marrow microenvironment was investigated *in vitro* and *in vivo*. In myeloma-bearing KaLwRij/5TGM1 mouse model, the BMP signalling inhibitor LDN-193189 (LDN) significantly improved bone volume ($P=0.003$) and reduced serum TRAP levels ($P=0.003$). LDN had no effect on overall tumour burden, however altered the niche-preference (endosteal vs. central marrow) of myeloma cells to favour the endosteal niche, reported to house the dormant clonal fraction (ratio endosteal: central marrow myeloma distribution 0.23 vehicle group, 0.37 LDN group, $P=0.034$). In early disease, serum hepcidin correlated with disease burden ($r^2=0.55$ $P=0{\cdot}03$), and was partly normalised by LDN. Tumour BMP signalling activity, measured by expression of BMP response genes id1 and smad6, increased tenfold when myeloma cell lines were cultured in contact with bone marrow stromal cells. This effect was abrogated by LDN. Expression of rankl was decreased by LDN in stromal cells from myeloma-bearing mice ($P=0.03$). In summary, BMP inhibition improves myeloma bone disease potentially by reducing RANKL levels thereby reducing osteoclast activity. The additional benefit of hepcidin reduction could alleviate the inflammatory anaemia of myeloma. If LDN also alters niche preference in favour of endosteal-sited dormancy, it could have use in prolongation of quiescent phases, e.g. MGUS and post-treatment remission. Taken together, our results demonstrate the potential for inhibition of BMP signalling for the treatment of myeloma and the associated bone disease.

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CABS.OC3.1

Blockade of C5aR impairs tumor-induced osteoclastogenesis preventing bone metastasis colonization in lung cancer

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C5aR is a membrane-associated receptor for C5a, a potent immune mediator generated after complement activation. C5aR expressed in tumor infiltrating immune cells creates a favorable microenvironment for tumor progression. However, the expression of C5aR by cancer cells and its contribution to their malignant phenotype is poorly understood. Immunohistochemical analysis revealed that high levels of C5aR in human lung tumors were associated with poor survival ($P=0.005$) and recurrence-free survival ($P<0.001$) in a cohort of 76 patients. Lentiviral shRNA-mediated C5aR silencing in A549 lung adenocarcinoma cells showed unaltered growth kinetics *in vitro* and *in vivo*. However, intracardiac inoculation (i.c.) of C5aR-silenced cells led to a substantial reduction in skeletal metastatic burden ($P<0.001$), as assessed by bioluminescence imaging, and the presence of osteolytic lesions ($P<0.001$), evaluated by X-ray and μCT imaging and histological analysis. A decreased tumor cell growth was detected in vivo (Ki-67) ($P<0.05$). These findings were validated in H460 large-cell lung carcinoma cells using an identical approach. Similarly, pharmacological blockade using a specific inhibitor of C5a significantly reduced the osseous metastatic activity of A549 cells after i.c. inoculation. This effect was associated with decreased bone colonization, since intratibial injection of shC5aR cells showed a reduction in tumor burden as compared to control animals. Interestingly, a reduced $TRAP^+$ osteoclast staining was detected in bones derived from shC5aR inoculated animals ($P<0.05$). These results were correlated with a marked reduction in osteoclastogenic activity ($TRAP^+$) induced by conditioned medium from shC5aR cells cultured alone or co-cultured with ST-2 bone marrow-derived stromal cells ($P<0.001$). Furthermore, C5aR also enhanced metalloproteolytic activity, and migration and invasion capabilities in vitro. These data indicate that C5aR disruption abrogates osteoclatogenic activity, critically impairing osseous colonization. We suggest that C5aR could represent a clinically relevant factor in lung cancer prognosis and a potential therapeutic target.

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CABS.OC3.2

Osteoblasts inhibit the immune response against cancer

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Osteoblasts line the inner surface of the bone and are located in close proximity to the bone marrow, where hematopoiesis takes place. Osteoblasts produce several cytokines that affect various steps of hematopoiesis, and produce fibronectin isoforms that affect cell differentiation. Little is known however on whether osteoblasts modulate the immune response. Our aim was to evaluate the role of fibronectin originating from the osteoblasts on hematopoiesis and the immune response to cancer.
Using conditional deletion of fibronectin in differentiating osteoblasts (Col-alpha1(I)-cre/+_fibronectin$^{floxed/floxed}$; cKO) we detected a decrease in the percentage of myeloid cells (CD11b+) by 28% ($P<0.05$) in the bone marrow. In transplantation experiments, followed by cocultures of bone marrow with control (CT) and cKO osteoblasts, we confirmed that the decrease in myeloid cells was only present when using cKO osteoblasts. Addition of three isoforms of fibronectin produced by osteoblasts allowed the attribution of the change in myeloid cells to the production of the isoform called EDA-fibronectin by the osteoblasts.
Cancer growth of the breast cancer cell line MDA-MB-231/luc in cKO was diminished as evidenced by increased survival (by 14%, $P<0.05$) and decreased growth (by 18–35%) after intracardiac and intratibial injection.
In order to determine whether this was due to a change in myeloid cell function we isolated these cells from bone marrow, determined their cytokine profile and found that arginase-1 was increased in cells exposed to EDA. Adoptive transfer experiments showed that EDA-fibronectin stimulated arginase-1 expression in myeloid cells and hence cancer growth, and inhibiting EDA effects by interfering with the mediating receptor, or preexposure to arginase-1-inhibitor prevented the EDA-mediated enhancement of cancer growth.

In summary, EDA fibronectin produced by the osteoblasts increases arginase-1 expression which in turn suppresses the immune response against cancer. Preventing osteoblastic-EDA effects, on the other hand, improves the immune response against cancer and diminishes its growth. These findings suggest new possibilities in the modulation of the immune response against cancer.

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CABS.OC3.3

Visualizing the tumor immunity in living bone marrow by intravital 2-photon imaging

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Within a living organism, the adaptive immune system, e.g. cytotoxic T lymphocytes (CTLs), induce apoptosis in tumor cells, and therefore limit tumor development. Bone marrow is a mysterious hidden place for different tumor cells and the anti-tumor immunity in the bone marrow is important because the specific microenvironment had been considered to contribute to escape of tumor cells from immune attack. However, the practical mechanism of anti-tumor immune elimination by CTLs in the bone marrow is still unclear. In this study, to elucidate how the CTLs response against tumor cells in bone marrow, we observed the interaction of leukemic cells and CTLs as well as CTL-induced apoptosis in the bone marrow using an intravital two-photon microscopy.

For visualizing the adaptive immune system, we used a fluorescent FRET-probe (SCAT3.1 FRET probe) that allows us to monitor caspase-3 activity and also used the ovalbumin-OT-I CTL system for inducing antigen specific T cell immunity.

First we visualized anti-tumor immune responses facilitated by CTLs *in vitro*. In results, leukemic cell death was dependent on the total cell number of CTLs. CTLs induced leukemic cell death by cell-cell contact; capturing leukemic cells, inducing caspase-3 activation and facilitating cell lysis. The CTLs took 3.5 hours on average to induce caspase-3 activation after capturing, and 2.4 hours on average to lysis the tumor cell after caspase-3 activation.

Secondly in mouse leukemia model, we observed that CTLs inducing apoptosis in leukemic cells upon directed cell-cell contact in the bone marrow by intravital 2-photon imaging. CTLs contact to leukemic tumor cells and induce caspase-3 activation, and remain in cell-cell contact to apoptotic leukemic cells until they undergo cell lysis.

Visualizing how tumor cells are killed by CTLs *in vivo*, especially in bone marrow, offers new perspectives for understanding anti-tumor immune elimination and the mechanism of escape from immune attack.

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CABS.OC3.4

Anti-sclerostin treatment prevents multiple myeloma bone disease and reduces tumour burden

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Multiple myeloma is characterized by extensive bone marrow tumour and destructive osteolytic lesions. Both increased bone resorption and suppressed bone formation result in lesions and pathological fractures. Anti-resorptive therapies prevent further bone loss but patients continue to fracture, arguing for new therapies which increase bone strength. Anti-Sclerostin (Anti-Scl) is a potent stimulator of bone formation, is currently in clinical trials for osteoporosis, however it is yet to be explored in the setting of myeloma. We demonstrate that Anti-Scl (100 mg/kg weekly i.v.) prevents bone loss, increases bone strength and suppresses myeloma growth in murine myeloma. MicroCT analysis demonstrated a 29% loss in trabecular bone volume ratio (TbBV/TV) and 15% loss in cortical bone volume (CtBV) in vertebrae of mice bearing 5TGM1/eGFP murine myeloma cells ($P<0.01$). This loss in bone structure led to a 34% reduction in vertebral peak load under compression ($P<0.01$). Anti-Scl treatment prevented the structural bone loss seen in 5TGM1 burdened mice, increasing TbBV/TV by 29% and CtBV by 18%, thereby increasing vertebral peak load by 47% ($P<0.01$). Anti-Scl treatment did not alter tumour burden in the 5TGM1 model, whereas, in the human MM1S xenograft model, it suppressed tumour growth ($P<0.05$). Our results show that Anti-Scl treatment prevented the development of myeloma bone disease, improving bone microarchitecture and bone strength. This demonstrates the potential for Anti-Scl treatment in addressing bone loss and fractures in patients with myeloma. Furthermore, suppression of tumour growth with Anti-Scl treatment suggests a dual action for this agent in myeloma, limiting tumour growth whilst improving skeletal integrity.

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CABS.OC3.5

Targeting skeletal metastatic breast cancer with bisphosphonic matrix metalloproteinase-2 inhibitors

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Breast to bone metastasis is a common incurable event during breast cancer progression. Identifying the molecular mechanisms at play is vital for the development of new therapies. Matrix metalloproteinases, such as MMP-2, are overexpressed in the bone metastatic microenvironment. Genetic ablation of MMP-2 demonstrated its importance of in driving osteolytic bone metastatic breast cancer and support the rationale for the development of a highly specific MMP-2 inhibitor for the eradication of active bone metastatic breast cancer.

Given that previous broad-spectrum MMP inhibitor (MMPI) trials were unsuccessful due to dose limiting systemic side effects, we utilized a novel chemical approach to synthesize bone seeking MMP inhibitors (BMMPIs) on a bisphosphonic backbone, with specificity for MMP-2 ($IC_{50}=140$ nM). *In vitro*, we tested the effect of BMMPIs on the viability of the major cellular components of the cancer-bone microenvironment (breast cancer cells, osteoblasts and osteoclasts). *In vivo*, mice received intratibially luciferase expressing breast cancer cells and were then treated with vehicle, zoledronate or BMMPIs (1mg/kg). Tumor growth was determined via luminescence quantitation. Cancer induced bone disease was measured *ex vivo* by μCT, Xray and histomorphometry. MMP activity *in vivo* and *ex vivo* was determined via specific activatable MMP probes. We observed that BMMPIs significantly impacted the viability of breast cancer cells and osteoclasts *in vitro* compared to control. *In vivo* BMMPIs significantly reduced the growth of bone metastatic breast cancer and MMP activity compared to control and zoledronate. *Ex vivo* analysis also illustrated the significant beneficial effects of the BMMPIs in reducing the size of osteolytic lesions (up to 80% by μCT; $P<0.05$).

In conclusion, MMP-2 specific BMMPIs prevent bone metastatic breast cancer growth by impacting cancer cell viability and cancer induced osteolysis. We predict that BMMPIs could be translated to the clinic for the treatment and eradication of bone metastatic breast cancer.

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CABS.OC4.1

Muscle dysfunction in immune competent mice with osteolytic breast cancer in bone is associated with skeletal muscle oxidation of RyR1

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Cancer-associated muscle weakness is an important paraneoplastic syndrome for which there is currently no treatment. Human breast cancer bone metastases (MDA-MB-231 cells in immune deficient mice) induce extensive bone destruction, leading to the release of TGF-β from the bone matrix. We have previously shown that bone-derived TGF-β is responsible for muscle weakness in this model. Mechanistically, TGF-β signaling increases the expression of NADPH oxidase 4 (Nox4), which oxidizes the skeletal muscle ryanodine receptor/calcium release channel (RyR1) leading to sarcoplasmic reticulum (SR) calcium leak. To determine if similar bone-muscle interactions occur with tumor-induced bone destruction in immune competent mice, we injected mouse breast cancer cells (4T1; 100,000 cells) into the tibia of Balb/C mice. The mice rapidly developed osteolytic tumors, weakness and cachexia. Fat and lean content

(assessed by DXA) both decreased in mice with 4T1 osteolytic lesions. All *ex vivo* muscle measurements and molecular analyses were performed on muscle from the limb contralateral to the tumor. Muscle weights ($P<0.0001$) and fiber cross-sectional area ($P=0.005$) were lower in mice with osteolytic lesions, an effect potentially driven by the increased expression of the atrophy-associated E3 ubiquitin ligases MuRF1 ($P=0.004$) and atrogin-1 ($P=0.004$). *Ex vivo* contractility of the extensor digitorum longus (EDL) muscle showed a significant reduction ($P<0.0001$) in specific force in tumor bearing mice compared to controls. Skeletal muscle from tumor bearing mice had higher levels of SMAD3 phosphorylation and Nox4 expression, consistent with increased TGF-β signaling. Finally, the calcium release channel RyR1 was oxidized and depleted of the stabilizing subunit calstabin1. These results echo the biochemical signature we have reported in immune deficient mouse models. Therefore, these data indicate that the phenotype and underlying mechanisms of skeletal muscle weakness in mice with osteolytic breast cancer in the bone are not altered by the immune status of the mice.

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CABS.OC4.2

Bisphosphonates prevent osteolysis and muscle weakness in aromatase inhibitor-treated mice with breast cancer bone metastases

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Up to half of women treated with an aromatase inhibitor (AI) for breast cancer develop muscle weakness, bone loss, and joint pain. Moreover, an elevated state of osteoclastic bone resorption has been shown to prime the bone microenvironment in ways that accelerate metastatic growth. We hypothesized that AI-induced bone loss could increase breast cancer progression in bone and exacerbate muscle weakness associated with bone metastases. Four-week female athymic nude mice underwent ovariectomy (OVX) or sham surgery and were treated with vehicle or AI (letrozole; 10 μg/day) ±bisphosphonate (zoledronic acid, ZA; 0.1 μg 3×/week; $n=20$/group). At week three, bone volume was reduced in OVX-AI mice (−67%, $P<0.0001$) and increased in OVX-AI-ZA mice (304%, $P<0.0001$) relative to sham-vehicle. Mice were then inoculated with 100,000 MDA-MB-231 human breast cancer cells into the left cardiac ventricle and followed for cancer progression. Since MDA-MB-231 cells are ER-negative, effects of estrogen deprivation on the tumor can be attributed to changes in the microenvironment. Five weeks after inoculation, osteolytic lesion area by X-ray was increased in OVX-AI mice (14.8 *vs* 7.1 mm^2, $P<0.05$) and reduced in OVX-AI-ZA mice (3.1 *vs* 7.1 mm^2 $P<0.05$) relative to sham-vehicle. Tumor burden in bone was increased in OVX-AI mice relative to sham-vehicle (11.6 *vs* 6.2 mm^2, $P<0.05$) and relative to OVX-AI-ZA mice (6.6 mm^2, $P<0.05$). Finally, muscle-specific force of the extensor digitorum longus was reduced in OVX-AI mice relative to sham-vehicle (399 *vs* 451 kN/m^2, $P<0.05$), and unchanged in OVX-AI-ZA (436 kN/m^2, $P=$ns). In summary, prevention of AI-induced osteoclastic bone resorption attenuated the development of breast cancer bone metastases and improved muscle function in mice. These findings highlight the bone microenvironment as a powerful modulator of tumor growth locally and muscle function systemically. Further studies are necessary to determine the relative contribution of estrogen deficiency, bone loss, and direct AI toxicities to impaired muscle function in this model.

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CABS.OC4.3

***In vitro* mechanotransduction of osteosarcoma cells**

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Osteosarcoma is a heterogeneous tumor from the mesenchymal lineage, and is the most common form of primary bone cancer. Normally, lesions contain undifferentiated cancer stem cells (CSCs) that support uncontrolled growth/proliferation, and pre-osteoblasts that form excessive amounts of immature bone. CSCs are characterized by high expression of the transcriptional regulators Sox2 and yes-associated protein (YAP) that are essential for tumorigenicity. YAP is restrained by the Hippo pathway, via its phosphorylation and nuclear exclusion, whereas Sox2 acts as an inhibitor of this pathway while also maintaining cell "stemness." Interestingly, YAP activity is also mechanosensitive, and the main transcriptional activator that responds to changes in substrate stiffness. In healthy bone physiology, mechanical signals are vital for homeostasis, and the primary source of mechano-stimulation is shear-stress induced via interstitial fluid flow. Our objective was to establish the effect of fluid flow induced shear-stresses (FSS) on osteosarcoma tumorigenicity and heterogeneity. Specifically, we investigated whether FSS regulates YAP activity and osteogenic gene expression in osteosarcoma derived cells. Tumor cells (mOS482) were obtained from spontaneous osteosarcoma occurring in mice with bone specific knockouts of pRb and p53. Monolayer cell-cultures were exposed to physiological FSS in a parallel-plate flow device, while controls experienced no flow. Cells were isolated and subcellular location of YAP and osteogenic gene expression were quantified using immunocytochemistry and qPCR, respectively. Nuclear YAP and Osterix expression increased due to FSS ($P=0.01$), while two other osteogenic genes (Runx2, -Osteocalcin) increased slightly, but did not reach significance. FSS also increased osteoprotegerin expression (OPG, $P=0.03$), however the ratio between OPG and RANKL did not change. These findings demonstrate that osteosarcoma cells are mechano-sensitive and mechanical signals increase their expression of osteogenic genes. This concept may present novel paradigms where tumor cell behavior and fate could be modulated by targeting its physical micro-environment.

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CABS.OC4.4

3D tissue engineered constructs for modeling tumor-induced bone disease

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While the importance of interactions between bone and tumors is well-established, the mechanism by which the physical bone microenvironment regulates disease progression is limited by the lack of suitable *in vitro* models. We have designed 3D Tissue Engineered Constructs (TECs) using microCT imaging in tandem with inkjet 3D printing technology that recapitulate the mechanical and morphometric properties of trabecular bone. 3D-printed TECs exhibited no significant differences in bone morphometric parameters compared to the human femoral head, tibial plateau, and lumbar spine templates from which they were prepared ($P<0.05$). The substrate modulus of the TECs was 266 MPa, which is within the reported range for trabecular bone (93–266 MPa). Culture of rat MSCs on the bone-like TECs exhibited a significant ($P<0.05$) 1.7-fold increase in mineralization compared to collagen-like TECs. Culture of bone-metastatic MDA-MB-231 breast cancer cells on bone-like TECs in a perfusion bioreactor showed a significant ($P<0.05$) >5-fold increase in expression of integrin beta 3, Gli2, and PTHrP compared to collagen-like TECs. Importantly, drug response differed remarkably when tumors were cultured on bone-like 3D TECs compared to tissue culture well plates. When MDA-MB-231 cells were treated with the integrin inhibitor Cilengitide or the TGF-beta receptor kinase inhibitor SD208 in 2D culture, expression of Gli2 and PTHrP significantly decreased two to three fold ($P<0.01$). However, treatment with these drugs did not significantly reduce Gli2 or PTHrP expression on 3D bone-like TECs. In contrast, treatment with the Gli2 inhibitor GANT58 significantly reduced both Gli2 and PTHrP expression >3-fold ($P<0.01$) in both 3D and 2D. These findings suggest that targeting factors downstream of cell receptors may be an effective approach to blocking establishment of tumors in bone. The TEC approach highlights the important contribution of the physical bone microenvironment for studying tumor and bone interactions and testing inhibitors of tumor-induced bone disease.

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CABS.OC4.5

New models of breast and lung cancer bone metastases for preclinical efficacy testing

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In advanced ER+ve breast cancer, the propensity of bone involvement is 85%. Similarly in advanced lung cancer, 30–40% of patients develop bone metastases,

and as recent advances in lung cancer therapies improve survival, the number of patients living with bone metastases is expected to increase. At the same time there is a paucity of especially ER+ and osteoblastic animal models available. We present herein the development of four mouse models of breast and lung cancer suitable for screening of new therapies.
Breast cancer cell lines BT-474 and MFM-223 represent ER+, i.e. luminal B, and basal subtype with AR expression, respectively. NSCLC cell line H226 originates from squamous cell carcinoma and H322 from adenocarcinoma of the lung. Cells were inoculated in the tibia of immunodeficient female mice. Half of the BT-474 inoculated mice had a s.c. slow release 17-beta estradiol pellet. The formation of bone lesions was monitored by X-ray imaging. For H226 transfected with luciferase, tumor growth was also followed by bioluminescence imaging (BLI). Finally, tumor growth and type of bone lesion was confirmed by histology. Bone lesions occurred in 100% and 90% of animals with or without hormonal supplementation, respectively, four weeks after inoculation of BT-474 cells. Bone lesions were detected earlier in mice with estradiol pellet and were of lytic type, whereas bone lesions in mice without hormonal supplementation were osteoblastic. For MFM-223, bone lesions were observed 4–6 weeks after inoculation in 60–70% of the animals. With both lung cancer cell lines, all mice developed bone lesions detectable already two weeks after inoculation. H226luc cells developed osteoblastic-mixed lesions and H322 cells induced lytic lesions. Very interestingly, H226luc cells also formed lung metastases in all animals, as evidenced by BLI.
New osteoblastic and osteolytic bone lesion models representing different subtypes of breast and lung cancer were successfully established.

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Oral Poster Talks

CABS.OP1.1

Osteoblastic and osteolytic bone metastases induce divergent angiogenic responses

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Cancer cell growth is dependent on the microenvironmental support. 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 types of 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 xenograft models of human osteoinductive PCa cells and of pro-osteolytic PCa and MCa cells.
The stroma transcriptome of osteoblastic bone metastases (OB-BMet) differs substantially from that of osteolytic bone metastases (OL-BMet). Although, the biological process "angiogenesis/vasculogenesis" dominates in both transcriptomes, the "vascular/axon guidance" process is manifest only in the OL-BMet. Consistently with this, the types of vessels are markedly different between the two types of metastatic lesions. In OB-BMet angiogenesis is characterized by sinusoidal morphology and marker expression of osteogenesis-coupled "H-vessels" that have been associated to the expanding hematopoietic stem cell (HSC) compartment in the marrow. In contrast, in OL-BMet angiogenesis is denoted by vessel morphology and marker expression specific for arterioles. These vessels have been associated with the maintenance of HSC quiescence. Remarkably, the stroma reaction in OL-BMet is also marked by a hyper-recruitment of mesenchymal stem cells/vascular smooth muscle cells, likely to be a major source of pro-osteolytic cytokines and of inhibitors of bone formation. This is also paralleled by intratumoral neurite ingrowth.
In conclusion, osteoinductive and pro-osteolytic cancer cells trigger divergent types of angiogenesis, representing functionally different HSC niches and, thus, different growth support requirements. This may imply the need for a differential anti-angiogenic strategy for interfering with tumour growth in osteoblastic and osteolytic lesions.

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CABS.OP1.2

Roundabout receptors: new actors in bone metastatic niche formation

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Roundabout receptors are crucial regulators of vessels and axons growth during development. A comparative transcriptomic study between the MDA-MB-231 breast cancer cell line and its osteotropic subpopulation, MDA-B02 cells, has shown an overexpression of ROBO4 receptor. Interestingly, a high ROBO4 expression in primary tumour from patients with breast cancer correlates with poor prognosis and increased risk of bone metastasis relapse. Inoculation of ROBO4 depleted cells in the mammary fat pad of mice led to a 50% reduction in tumour burden and a decrease of osteolytic lesions after injection of ROBO4-depleted tumour cells into caudal artery of mice. In vivo intra-tibial injection of ROBO4-depleted cells or MDA-B02 cells first incubated with an anti-ROBO4 antibody led to a decrease of the incidence and extent of tumour cell engraftment in the bone marrow. Altogether these results let us hypothesize that ROBO4 might facilitate the migration and engraftment of tumour cells in the bone marrow. *In vitro*, the co-culture of MDA-B02 cells with osteoblastic cell line MC3T3-E1 generated an increase in SLIT2 production, the ROBO receptor ligand, by MC3T3-E1 cells. Further, when we used an anti-ROBO4 or anti-SLIT2 antibody, MDA-B02 cell adhesion to MC3T3-E1 cell monolayer was dramatically reduced. These results suggest that anchoring of tumour cells in bone was driven by an interaction between ROBO4-expressing cancer cells and bone marrow osteoblasts. Furthermore, SLIT2 was involved in this interaction. Our findings suggest the use of an antibody directed against ROBO4 could lead to the development of innovative therapies to prevent bone metastatic niche formation.

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CABS.OP1.3

Cripto/Grp78 drive the metastatic phenotype in 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 patients with advanced disease at autopsy. PCa can progress from treatable androgen-dependent stage to castration-resistant stage with distant metastases for which novel therapeutic targets and strategies are urgently needed. Here we identify the cell surface/secreted oncoprotein Cripto as a potential target for the diagnosis and treatment of metastatic prostate cancer.
We show that high expression levels of Cripto correlate with poor survival in stratified risk groups of prostate cancer patients and demonstrate that Cripto and its signalling partner Grp78 are highly expressed in prostate cancer bone metastases.
Furthermore Cripto and Grp78 are expressed at substantially higher levels in the metastatic ALDHhigh subpopulation of stem-like PC-3M-Pro4luc2 prostate cancer cells compared to non-metastatic ALDHlow differentiated tumor cells. In order to mimic the endosteal HSC niche *in vitro*, we cultured the highly osteotropic PC-3M-Pro4luc2 cells with differentiated primary human osteoblasts. This strongly induces CRIPTO and Grp78 expression in the prostate cancer cells and it selectively increases the size of the ALDHhigh subpopulation relative to the ALDHlow cells. Additionally, Cripto or Grp78 knockdown decreases cell proliferation, migration, clonogenicity and the size of the metastasis-initiating ALDHhigh subpopulation. Moreover, Cripto knockdown reduces the dissemination and invasion of PC-3M-Pro4luc2 cells in a zebrafish model and strongly inhibits bone metastasis in a preclinical mouse model.
Taken together, our findings highlight a functional role for Cripto and Grp78 in prostate cancer metastasis and suggest that targeting Cripto /Grp78 signaling may have significant therapeutic potential.

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CABS.OP1.4

p62-ZZ domain signaling inhibition prevents MM cell-induced epigenetic repression at the *Runx2* promoter and rescues osteoblast differentiation

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Multiple myeloma (MM) bone disease is characterized by lytic bone lesions that contribute to patient morbidity and mortality after patients are in complete remission. The mechanisms mediating this long-term osteoblast (OB) suppression are poorly understood. We hypothesized that MM cells induce epigenetic changes at the *Runx2* promoter in preOB bone marrow stromal cells (BMSC). We demonstrated that Gfi1, a transcriptional repressor of *Runx2* that is induced in BMSC by MM, directly binds to the *Runx2* promoter, recruiting chromatin corepressors and inducing epigenetic repression of *Runx2* in preOB, thereby preventing OB differentiation.

We reported that p62 (sequestosome-1) in BMSC is critical for the formation of MM-induced signaling complexes that mediate OB suppression, and found that an inhibitor of the p62 ZZ domain, XRK3F2, blunted MM cell-induced *Runx2* suppression and *Gfi1* induction in murine preOB. *In vivo*, XRK3F2 induced new bone formation and remodeling in the presence of high tumor burden without altering bones without tumor.

We tested if XRK3F2 prevents the Gfi1-mediated epigenetic suppression of *Runx2* observed following MM exposure. ChIP analysis of murine preOB exposed to MM $\pm$ XRK3F2 demonstrated that XRK3F2 prevented MM-induced *Runx2* promoter Gfi1 occupancy, recruitment of the chromatin corepressor HDAC1, and histone de-acetylation. Coculture experiments using human MM cells and murine preOB showed that XRK3F2 both prevents and reverses *Gfi1* upregulation. Importantly, long-term culture of primary MM patient BMSC with XRK3F2 increased acetylation at the *Runx2* promoter, allowing rescued osteogenic differentiation and mineral deposition.

We conclude that XRK3F2 blocks MM-induced signaling, reducing recruitment of Gfi1 and its corepressor HDAC1 to the *Runx2* promoter, and preventing MM-induced epigenetic suppression of *Runx2*. These results suggest that targeting p62-ZZ as a therapeutic strategy in MM may reverse *Gfi1* upregulation, rescuing MM-induced epigenetic suppression of *Runx2* in BMSC and healing MM bone lesions.

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CABS.OP2.1

Antagonizing Mir-218 prevents breast cancer-induced osteolytic disease

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Signaling pathways crucial in bone development, including Wnt, are also upregulated in breast cancer cells to promote tumor growth in the skeleton, a process known as osteomimicry. Thus, we hypothesized that bone metastatic tumor cells also aberrantly express osteogenic miRNAs to support osteomimetic properties. We have previously shown that miR-218 is highly expressed in osteoblasts and promotes osteogenic differentiation. Interestingly, expression analysis revealed a significant up-regulation of miR-218 in bone metastatic MDA-MB-231 breast cancer cells compared to normal mammary epithelial cells. Furthermore, miR-218 was highly expressed in bone metastases biopsies from breast cancer patients, suggesting a positive role in bone metastasis. Indeed, delivery of miR-218 in MDA-MB-231-Luc cells promoted tumor growth in the bone microenvironment *in vivo* whereas inhibition of miR-218 impaired tumor growth. Signaling pathway analyses revealed a positive correlation between aberrant miR-218 expression and activation of Wnt signaling, demonstrated by reporter assays and expression of Wnt transcriptional mediators. Mechanistically, miR-218 targeted Wnt inhibitors Sclerostin and sFRP-2 and thus, delivery of miR-218 further enhanced while inhibition of miR-218 decreased Wnt activity. Importantly, the miR-218-induced expression of metastasis-related genes including bone sialoprotein, osteopontin and CXCR-4 was abolished by inhibition of Wnt signaling, indicating a Wnt-dependent regulation. Furthermore, PTHrP, a key cytokine promoting cancer-induced osteolysis was up-regulated in breast cancer cells by miR-218 in a Wnt-dependent manner. Consequently, conditioned medium from miR-218 expressing breast cancer cells increased Rankl in osteoblasts and supported osteoclast differentiation in osteoblast-osteoclast co-cultures. Importantly, antagonizing miR-218 reduced the expression of PTHrP and Rankl, inhibited osteoclast differentiation *in vitro* and *in vivo*, and prevented the development of osteolytic lesions in a preclinical metastasis model. In conclusion, we propose that miR-218 activates Wnt signaling to enhance metastatic properties of breast cancer cells and cancer-induced osteolytic disease. Therefore, antagonizing miR-218 represents a novel therapeutic intervention to prevent disease progression.

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CABS.OP2.2

Mesenchymal stromal cells promote osteosarcoma stemness and migratory potential via IL-6 secretion

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Osteosarcoma (OS) is an aggressive malignancy characterized by a high relapse rate despite multiagent chemotherapy. As for other cancers, OS microenvironment contributes to tumor initiation, growth, and metastasis. We consider bone marrow mesenchymal stromal cells (MSC) as a major nontumor component of OS microenvironment, and have previously found that the interaction between MSC and tumor cells is bidirectional, i.e. tumor cells can modulate their peripheral environment that, in turn, becomes more favourable to tumor growth through metabolic reprogramming (1). Here, we determined the effects of MSC on OS stemness and migration, two major features associated with an increased tendency to relapse and chemoresistance. The presence of MSC enhanced the number of floating spheres enriched in cancer stem cells (CSC) of the OS cell population. Furthermore, co-culturing with MSC stimulated the migratory capacity of OS via IL-6 secretion, and this effect was impaired by the neutralizing antibody Tocilizumab. Thus, MSC and OS-CSC exploit a vicious cycle in which the presence of CSC stimulates MSC cytokine secretion, in turn increasing stemness, proliferation, migration, and metastatic potential of CSC. Furthermore, for the first time, we identified Met, an oncogene that we have observed frequently expressed in aggressive OS (2), and found relevant for the pathogenesis of this tumor (3), as a novel stem cell marker. Altogether, our data highlight the need for a comprehensive knowledge of the interplay between tumor and stroma, that also includes the stem-like fraction of tumour cells, in order to develop novel and more effective anti-cancer therapies.

1. *Oncotarget.* 2015 **6** 30453–30471.
2. *Oncogene*. 1995 **10** 739–749.
3. *Cancer Res*. 2006 **66** 4750–4757.

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CABS.OP3.1

Abstract withdrawn

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CABS.OP3.2

Lysyl oxidase promotes survival and outgrowth of colon cancer cells in the bone marrow, enabling bone metastasis formation

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Lysyl oxidase (LOX) catalyzes the cross-linking of collagens and elastin in the extracellular matrix, thereby regulating the tensile strength of many tissues, such as in bone. In cancer, LOX plays a critical role in facilitating tumor growth and metastasis formation in soft tissues. In this study, we first showed by immunohistochemistry using patients' tumor specimens, that LOX was expressed in the desmoplastic tumor stroma of pairs of colorectal carcinomas and their matching bone metastases. Preclinical experiments showed that LOX overexpression in different colon carcinoma cells enhanced the formation of osteolytic lesions in animals by promoting both skeletal tumor burden and osteoclast-mediated bone resorption. Conversely, the pretreatment of animals with the LOX inhibitor β-aminopropionitrile or the silencing of LOX in colorectal carcinoma cells drastically reduced the formation of osteolytic lesions. Furthermore, we demonstrated that LOX was involved in the early nidation of tumor cells into the bone marrow. *In vitro*, LOX directly enhanced the attachment of colon cancer cells to type-I collagen, but not to fibronectin. LOX-overexpressing colorectal carcinoma cells were more prone to adhere to components of the osteoblastic niche, such as osteoblasts. Thus, LOX may promote engraftment of colon cancer cells in the osteoblastic niche. Tumor-derived LOX also promoted osteoclast differentiation by enhancing the secretion of osteolytic factors such as IL6. The activation of an IL6 autocrine loop led to cancer cell survival. In conclusion, our findings provide novel evidence that LOX endows colon cancer cells with the ability to thrive in the bone marrow microenvironment and stimulate osteoclast-mediated bone destruction.

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New Investigator Oral Communications

Abstract Presentations

NI1

Rictor plays a critical role in bone mass and strength with the involvement of Mtorc2 pathway in osteoblasts

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Mammalian target of rapamycin (mTOR) functions mainly in the form of two complexes, namely mTORC1 and mTORC2, which are distinct in their unique components, raptor and rictor. Here, we focused on bone phenotypes in mice with a specific deletion of rictor using a Cre recombinase gene whose expression was driven by the promoter of osteocalcin. All procedures involving mice were approved by the Institutional Animal Care and Use Committee of the local admin. DXA analysis showed a significant reduction in BMD of the $Rictor_{ob-/-}$ mice (53.5 mg/cm^2 vs 59.3 mg/cm^2, $P<0.001$). Furthermore, micro-computed tomography, histomorphometric, and molecular biological analyses revealed a marked impairment of the cortical bone growth, as well as minor changes in trabecular bone, of the $Rictor_{ob-/-}$ mice. Cortical tissue mass (1138.17 mg/ccmHA vs 1179.52 mg/ccmHA, $P<0.01$) and thickness (162.4 μm vs 193.6 μm, $P<0.001$) of the femoral mid-shaft were dramatically reduced, with unusual increases in porosity (0.69% vs 0.36%, $P<0.01$) and marrow area (0.98 mm^2 vs 0.91 mm^2, $P<0.05$) by micro-CT. These changes were associated with significantly decreased bone mechanical properties of the femurs as reflected by reduced peak load (15.03N vs 20.54N, $P<0.001$) and stiffness (43.47N/mm vs 58.98N/mm, $P<0.001$). Thinner trabeculae were found in the lumbar spine (25.81 μm vs 33.22 μm, $P<0.001$) with relatively normal structural indices of trabecular numbers and separation by histomorphometry. However, there were no significant changes in the trabecular bone of the distal femur by micro-CT. A lower rate of bone turnover was observed, as the consequence of the decreased individual osteoblast and osteoclast activities. Furthermore, osteoblast differentiation was reduced, with down-regulation of mTORC2 signaling activity as shown in primary cultures of osteoblasts that did not contain rictor. In conclusion, expression of rictor in osteoblasts is essential for the maintenance of normal bone modeling/remodeling and bone mass, especially for the normal accruement of cortical bone.

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NI2

Circulating microRNAs in postmenopausal women with osteoporosis and vertebral fractures

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Circulating microRNAs (miRNAs) are currently being investigated as novel biomarkers for osteoporosis and osteoporotic fractures. The aim of the present study was to investigate the differential expression of specific circulating micro RNAs known to regulate bone metabolism and homeostasis in postmenopausal osteoporotic women with and without vertebral fractures. For the analysis, miRNAs were isolated from the serum of 24 osteoporotic patients with at least one moderate vertebral fracture and 24 osteoporotic women without vertebral fractures. Twenty postmenopausal women with normal BMD and with no previous history of any kind of fracture were also included in the analysis as controls. From the 14 miRNAs that were selected we identified seven miRs, namely miR-21, miR-23a, miR-124, miR-2861, miR-29a, miR-29b, miR-29c that were significantly deregulated in the serum of osteoporotic patients compared to controls. Two of them (miR-124 and miR-2861) were significantly upregulated while miR-21, miR23a, miR29a, miR29b and miR-29c demonstrated a significantly lower expression in the serum of osteoporotic patients compared to controls. In the sub-group analysis in the osteoporotic group of patients, miR-21, miR-29a, miR-29b and miR-29c were significantly lower in osteoporotic fractured patients compared to osteoporotic patients without fractures. MiR-218, was upregulated in the fractured osteoporotic patients compare to non-fractured, but without reaching statistical significance. This study shows that the expression pattern of specific miRNAs in the serum of osteoporotic patients at increased risk for vertebral fractures may be used as a diagnostic tool for further optimizing fracture risk assessment.

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NI3

Tumor necrosis factor superfamily members in bone loss in men with end-stage chronic obstructive lung disease

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Aim
To assess the relationships of serum osteoprotegerin (OPG), receptor-activator of nuclear factor-κB ligand (RANKL) and tumor necrosis factor-alpha (TNF-α) superfamily with bone composition in end-stage COPD.
Methods
Body and bone composition, serum OPG, RANKL, TNF-α and its receptors (sTNFR I, sTNFR II), TNF-related apoptosis-inducing ligand (sTRAIL) levels were measured in 48 men end-stage COPD accepted for lung transplantation, and 36 male non COPD volunteers.
Results
OPG was lower in male COPD patients than in control whereas RANKL, TNF-α and its receptors were higher. No notably difference in the serum sTRAIL concentrations between the two groups. OPG directly correlated with FEV1% ($r=0.49$, $P=0.0005$), fat mass index ($r=0.46$, $P=0.001$), lumbar and femoral T-score ($r=0.653$, $P<0.0001$ and $r=0.686$, $P<0.0001$). Serum RANKL inversely associated with FEV% ($r=0.49$, $P=0.0004$), body lean mass ($r=-0.68$, $P<0.0001$), lumbar and femoral T-score ($r=-0.65$, $P<0.0001$ and $r=-0.56$, $P<0.0001$) but directly correlated with TNF-α ($r=0.52$, $P=0.0002$). A similar pattern of association with FEV1 was observed for sTRAIL, TNF-α and its receptors. OPG was inversely correlated with RANKL ($r=-0.56$, $P<0.0001$), TNF-a ($r=-0.62$, $P<0.0001$), sTRAIL ($r=-0.31$, $P=0.034$) and sTNFR-I ($r=-0.512$, $P<0.0001$). Using backward selection multivariable regression, increased serum TNF-α and RANKL were independently associated with lumbar bone loss (adjusted $R^2=0.61$) while RANKL and weight independently predicted femoral T-score (adjusted $R^2=0.564$). In addition, an increased level of serum RANKL and lowered serum OPG concentration were independently associated with reduced skeletal lean mass (adjusted $R^2=0.51$).
Conclusion
Our results suggest that serum RANKL levels are remained significantly associated with reduced pretransplant BMD in male COPD.
Acknowledgments
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Key words: osteoporosis, chronic obstructive pulmonary disease, TNF-α, osteoprotegerin, RANKL, bone metabolism
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NI4

The correlation between number and population of bone marrow endothelial progenitor cells with bone mass and bone metabolism in the elderly

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Objective
Endothelial progenitor cells (EPCs) have the potential ability to differentiate into vascular endothelial cells and osteoblasts for angiogenesis and osteogenesis, however, the correlation between number and population of bone marrow EPCs with bone mass and bone metabolism in elderly is unknown.
Methods
Trabecular bone were extracted from 11 patients with fragility fracture and eight patients with osteoarthritis during artificial hip replacement surgery, and EPCs separated from bone marrow were prepared for flow cytometry analysis. All the patients took DEXA scan, and bone metabolism detection. The impact of clinical data such as age, BMI, bone mass and bone metabolism markers on the number and population of bone marrow EPCs in the elderly were analyzed.
Results
There was no significant difference of age and BMI between fragility fracture patients and osteoarthritis patients. The total number of bone marrow EPCs and number of mature EPCs in fragility fracture patients were significantly less than that in osteoarthritis patients (0.48 ± 0.35 vs 1.80 ± 1.01, $P=0.001$; 52.28 ± 21.20

vs 77.13±19.15, $P=0.042$), and the bone mass of femur neck and total hip (0.54±0.14 vs 0.76±0.21, $P=0.021$; 0.65±0.14 vs 0.84±0.15, $P=0.026$) as well as serum 25(OH)D level (4.50±1.56 vs 23.80±2.88, $P=0.033$) in fragility fracture patients were significantly lower than those in osteoarthritis patients, however serum PTH lever (73.60±1.84 vs 32.20±0.98, $P=0.035$) was significantly higher in fragility fracture patients than that in osteoarthritis patients. There are significantly negative correlation between age with number of mature EPCs ($r=-0.594$, $P=0.015$), and positive correlation between bone mass in femur neck and total hip with number of mature EPCs ($r=0.847$, $P=0.008$; $r=0.925$, $P=0.034$), and negative correlation between bone mass in total hip with number of premature EPCs ($r=-0.817$, $P=0.047$). However, BMI, 25(OH)D and PTH didn't show any correlation with number of bone marrow EPCs.

Conclusion

Bone marrow EPCs could influence bone mass via regulating bone metabolism directly or indirectly in the elderly.

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NI5

Altered bone metabolism after high fat diet and exercise: role of Wnt signaling and insulin resistance

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High fat diet (HFD), obesity, and physical inactivity characterize the modern lifestyle. This can lead to coronary heart diseases and type 2 diabetes mellitus. Recent studies have shown that these conditions frequently are associated with poor bone quality. However, the molecular mechanisms are poorly understood. To analyze the effect of HFD and exercise (EX) on bone homeostasis, we fed 6-week-old C57BL/6 mice a high fat (60% fat) or standard chow diet for 10 weeks (10–20 mice per group). Half of each group had free access to running wheels. Ten minutes before sacrifice, 3*H*-2-Deoxy-D-glucose (2-DG) was injected into the retro-orbital vein to investigate 2-DG uptake in tissue. Afterwards, blood, muscle and bone samples were collected. Bone mass was analyzed using micro-computed tomography, serum parameters by ELISA, and 2-DG-uptake by liquid scintillation counting.

HFD increased body weight (+14%) in sedentary mice. This increase was prevented in mice that exercised (8 km/d). Blood glucose and plasma insulin levels were increased in both HFD groups (up to +59% and +111%, respectively), but were not changed by EX. HFD decreased the uptake of 2-DG in the muscle (−55%) and in the tibial bone marrow (−44%), whereas EX increased the uptake back to control levels. The femoral trabecular and cortical bone volume per total volume (BV/TV) was reduced after HFD (−49%) while EX did not affect the BV/TV in control or HFD mice. While EX had no influence on bone turnover in control mice, it increased bone turnover in mice on a HFD (CTX: +29%, P1NP: +23%, osteocalcin: carboxylated +51%, undercarboxylated +20%). The Wnt inhibitor Dickkopf-1 was also elevated in both HFD groups and was not affected by EX.

These data suggest that Wnt signaling and insulin resistance may play key roles in the altered bone metabolism induced by HFD and a sedentary lifestyle.

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NI6

Multi-potency and immunosuppressive activity of mesenchymal stromal cells derived from human induced pluripotent stem cells

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Tissue healing/reconstruction as well as exacerbated inflammatory diseases may benefit from stem cell based therapies. *Ex vivo* isolated tissue mesenchymal stromal cells (MSCs) displaying multi-potent activity and immune-regulatory functions were long ago proposed as therapeutic cells and already tested in many clinical assays. Nevertheless, their use may be restricted because of the few number that can be recovered from adult tissues, their limited *in vitro* expansion, and the absence of a full characterization. Other sources of well-defined and unlimited number of MSCs are needed, and MSCs derived *in vitro* from human Induced Pluripotent Stem (huIPS) cells would be a valuable tool for therapeutic approaches.

We developed a simple assay to generate huiPS–MSCs which were evaluated for their multi-potency and their immunosuppressive activity *in vitro* and *in vivo* in a humanized mouse model.

i) The huiPS–MSCs were phenotypically indistinguishable from tissue MSCs; they were capable of differentiation into osteoblasts, chondrocytes and adipocytes. Co-cultured with stimulated human T lymphocytes, huiPS–MSCs inhibited efficiently the T cell proliferation, switching the T cell cytokine polarization to a regulatory state.
ii) The *in vivo* immunosuppressive activity of huiPS-MSCs was evaluated using immune-deficient NOD/SCID/IL2rγKO mice in which human immune cells proliferate, infiltrating tissues. After treatment with huiPS-MSCs, the numbers of human circulating T lymphocytes, of those present in the peritoneal cavity and in the spleen were significantly reduced. Intracytoplasmic labelling of recovered T cells showed that untreated mice displayed high percentages of T cells producing inflammatory IFN and TNF cytokines. In contrast, in mice treated with the huiPS-MSCs, the proportion of inflammatory T cells was reduced, while that of T cells producing the anti-inflammatory IL-10 cytokine and expressing the FoxP3 was significantly increased.

We show that *in vitro* generated multi-potent immune-modulatory huiPS–MSCs may served as new therapeutic tolerogenic tools for inflammatory diseases or for reconstruction/healing processes.

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NI7

Exposure to chronic stress induces bone loss via glucocorticoid signalling in osteoblasts

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Chronic stress and depression are associated with alterations in the hypothalamic–pituitary–adrenal signalling cascade and considered a risk factor for bone loss and fractures. However, the mechanisms underlying the association between stress and poor bone health are unclear. Utilising a transgenic (tg) mouse model in which glucocorticoid signalling is selectively disrupted in mature osteoblasts and osteocytes ($HSD2^{OB}$-tg mice), the current study examines the impact of chronic stress on skeletal metabolism and structure.

Eight-week-old male and female transgenic mice and their wt littermates were exposed to chronic mild stress for the duration of 4 weeks. Stressors included restraining, exposure to hot and cold, tilted cages and overnight illuminations. At endpoint, L3-vertebrae and tibiae were analysed by micro-CT and histomorphometry, blood was collected for markers of bone turnover.

Compared to the non-stressed controls, exposure to chronic stress resulted in loss of vertebral trabecular bone mass in male WT mice but not in $HSD2^{OB}$-tg male littermates (wt: −15.9% tg: +2.8%, $P<0.05$). Bone loss in mice with intact osteoblastic glucocorticoid signaling was due to a decrease in trabecular number (wt: −14.3% tg: +0.8%, $P<0.01$) and an increase in trabecular separation (wt: +12.1% tg: +1.2%, $P<0.05$). While trabecular bone in the tibia was unaffected in stress-exposed WT and $HSD2^{OB}$-tg males, tibial cortical area (wt: −11.1% tg: +1.3%, $P<0.05$) as well as cortical area fraction (wt: −9.5% tg: −2.6%, $P=0.054$) were reduced in stressed WT but not in stressed $HSD2^{OB}$-tg male mice. Histomorphometry and measurements of serum TRAP5b revealed an increase in osteoclast activity in wild-type males following stress exposure, an effect that was absent in $HSD2^{OB}$-tg males. Interestingly, in female mice, both vertebral and long bone structural parameters remained unaffected by chronic mild stress.

We conclude that in male mice, bone loss during chronic mild stress is mediated via glucocorticoid signalling in osteoblasts and subsequent activation of osteoclasts.

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NI8

Bone marrow adipose tissue and bone turnover in postmenopausal osteoporotic women and the effects of raloxifene

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Background
In postmenopausal osteoporosis, a loss of bone volume due to increased bone turnover is accompanied by a higher volume of bone marrow adipose tissue (BMAT). If this static relationship is based on a functional relationship, BMAT is a potential target for treating osteoporosis. While it is known that estrogen can reduce BMAT, it is still unknown whether raloxifene – a selective estrogen receptor modulator – can also reduce BMAT.
Objective
To determine i) the correlation between BMAT and bone turnover in postmenopausal osteoporotic women and ii) the effect of raloxifene on BMAT.
Methods
We retrospectively analyzed paired iliac crest biopsies from 26 postmenopausal osteoporotic women enrolled in the MORE trial, both at baseline and after 2 years of treatment with placebo or raloxifene. All subjects received oral calcium and vitamin D_3. Standardized bone histomorphometry and BMAT parameters were measured in Goldner stained sections.
Results
At baseline, BMAT was correlated with bone volume ($R=-0.382$; $P=0.03$), but not with bone formation rate or osteoclast number. There was an increase in adipocyte density in the raloxifene group, while there was no change in adipocyte density in the in the placebo group (mean change from baseline: 34.1 s.d. 28.9 vs -3.5 s.d. 29.5 cells/mm^2; $P=0.003$). The adipocyte diameter did not change after raloxifene, while after placebo there was an increase in adipocyte diameter (mean change from baseline: -0.5 s.d. 2.8 vs 3.1 s.d. 5.1 μm; $P=0.03$).
Conclusion
This study suggests that BMAT is not correlated with bone turnover in iliac crest biopsies of postmenopausal osteoporotic women. Furthermore our results indicate that raloxifene does not reduce the volume of BMAT, but rather increases the number of bone marrow adipocytes while preventing an increase in the size of bone marrow adipocytes.

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NI9

Inflammatory conditions induces a new subset of osteoclasts that prime TNFα-producing CD4$^+$ T cells

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Chronic inflammatory diseases are characterized by a bone destruction mediated by an increased osteoclast (OCL) activity. OCLs are phagocytic cells arising from the myeloid lineage. Indeed, OCLs derive from monocytes (MN-OCLs) and, in an inflammatory context, they also derive from dendritic cells (DC-OCLs). Despite this origin, their role in the immune responses is still unclear. OCLs in steady state have been reported to act as antigen-presenting cells that activate CD8$^+$ regulatory T cells, revealing an immune suppressive function, but such function has never been studied in an inflammatory context.
Our aim was to address the effect of OCLs from different origin on CD4$^+$ T cell responses. We set up a unique procedure to purify OCLs on a cell sorter to analyze OCL specific immune function. Working with pure OCL populations, we showed that MN-OCLs and DC-OCLs have the same capacity to process and present antigens. On the other hand, DC-OCLs express high levels of inflammatory cytokines; they efficiently attract CD4$^+$ cells, and induce their differentiation into TNFα-producing T cells. In contrast, MN-OCLs are not efficient in attracting CD4$^+$ T cells; they induce their differentiation into regulatory T (Treg) cells and express higher levels of immunosuppressive IL-10. These results were confirmed using a murine model of colitis associated with an overactivation of OCLs, the Rag1$^{-/-}$ mice transferred with naive CD4$^+$ T cells. As MN-OCLs, OCLs from control mice induce CD4$^+$ T-reg cells, whereas those from colitic mice have the same inflammatory properties than DC-OCLs. Our results demonstrate that MN-OCLs are related with the BM tolerance in steady state, probably avoiding self-reactivity against the peptides continuously produced during bone resorption. In contrast, under inflammatory conditions DC-OCLs may induce inflammatory or autoimmune responses and participate to an amplification loop between bone destruction and inflammation.

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Oral Communications

Clinical trials and osteoporosis treatment

OC1.1

Efficacy of odanacatib in postmenopausal women with osteoporosis: subgroup analyses of data from the phase 3 long-term odanacatib fracture trial (LOFT)

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Odanacatib (ODN), a selective oral inhibitor of cathepsin K, is in development for the treatment of osteoporosis. In the primary efficacy analysis of the Phase 3, Long-Term ODN Fracture Trial (LOFT; NCT00529373), ODN significantly reduced fracture risk compared with placebo in postmenopausal women with osteoporosis. Pre-specified subgroup analyses evaluated the efficacy of ODN in patient subgroups.

Women aged $\geq$65 years, without baseline radiographic vertebral fracture (VFx) and total hip (TH) or femoral neck (FN) BMD T-score between -2.5 and -4.0, or with prior VFx and TH or FN T-score between -1.5 and -4.0, were randomised (1:1) to ODN 50 mg/week or placebo. All received vitamin D_3 (5600 IU/week), and calcium as required. Treatment effects on primary endpoints (new and worsening morphometric vertebral, hip, and non-VFx) were investigated in subgroups (including by age, bisphosphonate intolerance, prior radiographic VFx, baseline BMD).

Of 16,713 randomised women (387 centres in 40 countries), 16,071 were analysed. Baseline mean age was 72.8 years, 57% Caucasian, 46% prior VFx. Mean BMD T-scores were: lumbar spine (LS) -2.7, TH -2.4, FN -2.7. The risk reduction of ODN versus placebo for primary fracture endpoints was generally consistent across all subgroups. For morphometric VFx, relative risk reductions (RRR) for participants with or without prior VFx were 51 and 60%, respectively; for age groups $<$70 and $\geq$70 years, RRRs were 57 and 53%, respectively; and, by baseline LS BMD T-score tertiles (≥ -2.22; -3.25 to < -2.22; ≤ -3.25), RRRs were 58, 47 and 54%, respectively. In bisphosphonate-intolerant patients, the RRR for morphometric VFx, hip and non-VFx were 52, 42 and 17%, respectively, consistent with the overall population. Post-hoc statistics revealed no significant interactions.

In postmenopausal women with osteoporosis, the effect of ODN vs placebo was generally consistent among predefined subgroups in reducing the risk of new and worsening morphometric vertebral, hip and non-VFx.

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OC1.2

Acute effects of calcium supplements on blood pressure: results of a randomised cross-over trial

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Calcium supplements are associated with increased cardiovascular risk, but the mechanism by which this occurs is presently uncertain. In a secondary analysis of a trial examining the acute effects of calcium supplements, we found that blood pressure declined over 8 h in the control group, consistent with its diurnal rhythm, and that this decline was smaller in the calcium group [1]. To investigate these effects further, we carried out a randomised controlled cross-over trial of 40 healthy postmenopausal women. Women attended our clinic on two occasions at which they received a single dose of 1000 mg of calcium as citrate, or a placebo containing no calcium. Visits were separated by at least 7 days. Blood was sampled and blood pressure measured immediately before, and then 2, 4 and 6 h after, participants received each intervention. Ionised calcium increased from baseline after calcium citrate, and did not change after placebo. Blood pressure declined from baseline after calcium citrate and placebo. However, there was a significant difference between the changes in systolic blood pressure after calcium and placebo (ANOVA, $P=0.02$). The decline in systolic blood pressure from baseline was smaller after calcium compared with placebo by 4 mmHg at 2 h, 6 mmHg at 4 h and 9 mmHg at 6 h. A similar pattern was observed for diastolic blood pressure, although the differences were smaller (1–2 mmHg) and not significant. These findings confirm those of our previous trial, and suggest that in older adults the use of calcium supplements will result in blood pressures 4–9 mmHg higher in the 6 h after dosing. These changes in blood pressure, if repeated long-term, may contribute to the increased cardiovascular risk associated with calcium supplement use.

1. Bristow SM *et al.* (2015) Br J Nutr **114**, 1868–1874.

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OC1.3

Treatment with vitamin MK-7 prevents deterioration of trabecular bone

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Background

Clinical studies suggest that vitamin K2 prevents bone loss and may protect against fractures. Vitamin K is a cofactor in the carboxylation of Osteocalcin (OC). Carboxylated OC promotes mineralization of bone. The aim of the study was to investigate the effect of vitamin K2 on osteocalcin, bone mass and -quality.

Methods

We conducted a randomized placebo-controlled double-blinded clinical trial, in which 142 postmenopausal osteopenic women were treated with vitamin K2 (375 µg MK-7) or placebo. Both groups received vitamin D3 (38 µg/day) and calcium (800 mg/day). We measured bone markers and bone mineral density and -quality by DXA and HRpQCT. All results are given as changes after 12 months.

Results

Microarchitecture at the tibia changed differently between the groups; Trabecular number remained stable in the MK-7-group ($-0.6\pm8.2\%$), but decreased in the placebo-group ($-3.5\pm8.6\%$) ($P=0.04$), trabecular spacing did not change in the MK-7-group ($1.2\pm8.0\%$) but increased in the placebo-group ($4.5\pm9.7\%$) ($P=0.03$), and trabecular thickness was unaltered in the MK-7-group ($+0.7\pm7.7\%$) but increased in the placebo-group ($+4.0\pm8.7\%$) ($P=0.02$). Changes in microarchitecture in radius did not differ between groups.

Changes in aBMD did not differ between groups at either the lumbar spine or total hip ($P=0.23$ and $P=0.42$).

Undercarboxylated osteocalcin decreased in the MK-7-group ($-65.2\pm23.5\%$) compared to the placebo-group ($-0.03\pm38.5\%$) ($P<0.01$). Total OC decreased in the MK-7-group ($-18.8\pm13.9\%$) compared placebo-group ($-2.5\pm12.8\%$) ($P<0.01$). BAP increased in the MK-7-group ($+4.1\pm13.7\%$) compared to the placebo-group ($-1.3\pm12.7\%$) ($P=0.02$). Changes in P1NP and CTX did not differ between groups ($P>0.5$).

Conclusion

The change in undercarboxylated OC suggests increasing carboxylation of OC, which may indicate increased mineralization of bone. HRpQCT results suggest that vitamin MK-7 may prevent the age-related loss of trabeculae. However, there is no difference in changes in BMD. There is need for long-term investigations of the effect of vitamin K2 on bone.

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OC1.4

Relationship between total hip (TH) BMD T-score and incidence of nonvertebral fracture (NVFX) with up to 10 years of Denosumab (Dmab) treatment

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The relationship between BMD T-score and FX risk has not been established in patients receiving osteoporosis therapy. In the FREEDOM Extension study,

continuous DMAb therapy for up to 10 years increased BMD levels with no therapeutic plateau at lumbar spine or TH [Bone et al, ASBMR 2015]. Such improvements would only be meaningful if associated with FX reductions. We investigated the relationship between TH BMD T-score and NVFX in women who received DMAb during FREEDOM and those who continued to receive DMAb in FREEDOM Extension ($N=3612$; maximum of 10 years continuous treatment). A repeated-measures model was used to estimate each subject's BMD T-score time course during the entire study, and estimate BMD at each unique NVFX time among all subjects at risk. A Cox's proportional-hazards model was then fitted, with time to NVFX as response and TH BMD T-score time course a time dependent covariate. Higher TH BMD T-scores achieved during up to 10 years of DMAb therapy were associated with lower incidence of NVFX (Table); similar findings have been reported for treatment-naïve patients [Austin *JBMR* 2015]. For example, TH BMD T-scores of –2.5 and –1.5 were associated with 1-year NVFX incidence of approximately 3.0 and 2.0%, respectively. T-scores above −1.5 appear to have minimal impact on further reducing NVFX incidence. This inverse relationship was maintained regardless of age or prior FX (data not shown). Our findings suggest that BMD level achieved during treatment is more important for FX risk reduction than the magnitude of the change from baseline levels. Moreover, our findings support the concept that a specific T-score, perhaps in the range of −2.0 to −1.5, can be considered a therapeutic goal with DMAb treatment.

TH BMD T-score	Estimated 1-year NVFX incidence (%) [95% CI]
−3.0	4.03 [2.94, 5.11]
−2.5	3.01 [2.34, 3.67]
−2.0	2.38 [1.87, 2.89]
−1.5	2.00 [1.55, 2.45]
−1.0	1.78 [1.35, 2.21]
−0.5	1.68 [1.22, 2.14]

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OC1.5

Secular trends in prescription incidence of different anti-osteoporotic drugs in the UK population aged 50 years or above from 1990 till 2012

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Objective
To study the trends in prescription of different anti-osteoporotic drugs (AOD) in the UK population aged 50 years or above from 1990 till 2012.
Methods
Retrospective observational study using the CPRD data link representative for the total UK population. The incidence of prescription of a specific AOD was calculated by dividing the number of prescriptions by the total person-years (py) of follow-up.
Results
AOD prescription increased in women from 1990 till 2006 (from 2.3/10,000 py to 169.7/10,000 py), followed by a plateau of two years and subsequently a 12% decrease in the last four years, in spite of the introduction of ibandronate and strontium ranelate in 2005–2006. In men a steep increase from 1990 to 2007 (from 1.4/10,000 py to 45/10,000 py) was followed by a plateau. AOD prescriptions were mostly bisphosphonates (>90%). Until 2000 etidronate was the dominant bisphosphonate, after 2000 this was alendronate. Prescriptions for AOD increased with age, with the biggest increment in women, up to the age group of 85–89 in which the incidence was 248.9/10,000 py in women and 119.3/10,000 py in men. There were significant regional differences in the prescriptions for AOD with the highest incidence in women and men in Northern Ireland and the lowest in East Midlands (women), Yorkshire and the Humber region (men). The prescription of AOD also differed markedly between ethnic groups with the highest differences seen in women, were the incidence in black women is only half that in white and Asian women.
Conclusion
Overall AOD prescription incidence across the UK showed a dramatic increase from 1990 to 2009 in women, followed by a decrease from 2009 to 2012, while in men the increase stabilized. Almost all AOD prescriptions were bisphosphonates. Prescriptions incidences were substantially higher in women and increased with age. There were marked regional and ethnical differences.

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OC1.6

Standard QCT shows substantial underestimation of treatment effects on bone mineral density: impact of spatial resolution

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Quantitative Computed Tomography (QCT) can be performed with different protocols. We investigated whether selection of spatial resolution, reconstruction method, and scanner type have an impact on measured treatment effects for different bone compartments.
Methods
In a multi-center study, a group of 50 men, age 55.1 (range 25–78) undergoing 18 months of either osteoanabolic ($n=25$) or antiresorptive treatment ($n=25$) were scanned with QCT (120 kVp, 100 mAs, slice thickness 2.5–3 mm, in-plane pixel size 0.6 mm) and High Resolution QCT (HR-QCT: 120 kVp, 360 mAs, slice thickness 0.6–0.8 mm, reconstruction increment 0.3–0.4 mm, in-plane pixel size 0.16–0.19 mm). The protocol specified measurement at L1 for QCT and T12 for HR-QCT but due to fractures of T12 in seven patients HR-QCT and QCT were both obtained at L1 (thus impact of matching vertebral could be tested). We compared integral, cortical and trabecular bone mineral density (BMD) i) at baseline and ii) their changes during treatment (ΔBMD) as observed by QCT and HR-QCT, adjusting for vertebral level mismatch. All results are expressed as mean ± SEM.
Results
The table reveals large underestimation of treatment effects of 34–69% for standard QCT. The magnitude of underestimation was positively corrected with baseline BMD ($P<0.0001$ for all three compartments) and varied by scanner type/reconstruction kernel. Vertebral level mismatch had no additional independent significant influence.
Conclusion
Spatial resolution has a strong effect on CT-based BMD evaluations. HR-QCT reveals 45% (trabecular, integral) to 2.9 times (cortical) larger treatment effects for BMD and true treatment effects may be even larger due to spatial resolution limits of HR-QCT. This has major implications for the interpretation of QCT-based finite element modeling and for standardization of QCT studies.

Bone Compartment	HR-QCT BMD baseline	QCT BMD baseline	HR-QCT ΔBMD	QCT ΔBMD	Error BMD	Error ΔBMD
Trabecular	71.7±4.6	80.3±4.4	10.1±2.3	6.6±2.3	+12%	−35%
Cortical	271.2±8.1	171.4±.5	35.1±3.3	10.9±2.4	−37%	−69%
Integral	134.4±4.8	127.9±4.0	16.9±2.7	11.1±2.4	−5%	−34%

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Bone mass and bone strength Wnt signalling

OC2.1

Targeted deletion of Wnt1 in mesenchymal cells results in decreased bone mass and spontaneous fractures

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Wnt signaling is a major regulator of bone metabolism. We recently reported that mutations in WNT1 gene in humans cause early onset osteoporosis and severe osteogenesis imperfecta. To identify the cellular source and the mechanisms causing these severe phenotypes we generated and analyzed global and conditional Wnt1 knockout mice.
Heterozygous $Wnt1^{+/-}$ mice were viable and fertile but $Wnt1^{-/-}$ embryos were lost in utero. μCT analysis of tibiae at 12 weeks showed that BV/TV and Tb.N. were significantly decreased in $Wnt1^{+/-}$ male mice compared to controls. We had previously observed Wnt1 expression both in osteocytes and in hematopoietic cells. To bypass the embryonic lethality of $Wnt1^{-/-}$ mice and to identify the major source of Wnt1 in long bones, we targeted Wnt1 knockout to limb bud mesenchymal cells using Prrx1-Cre. All male $Wnt1^{-/-}_{Prrx1}$ mice exhibited spontaneous long bone fractures already by the age of 6 weeks. Trabecular BV/TV was decreased by 70% and Tb.N by 60% in proximal tibia of $Wnt1^{-/-}_{Prrx1}$ mice at 6 weeks by μCT. Significant but smaller reductions were observed at 12 weeks. Cortical thickness was decreased by 47% in $Wnt1^{-/-}_{Prrx1}$ mice in μCT. Histomorphometric analysis at 12 weeks showed significantly increased number of osteoclasts and eroded surface and decreased bone formation, despite unaltered osteoblast number or surface in $Wnt1^{-/-}_{Prrx1}$ mice. Expression of Wnt inhibitor Sclerostin was significantly lower in $Wnt1^{-/-}_{Prrx1}$ long bones. In vitro, $Wnt1^{+/-}$ calvarial cells showed impaired osteoblastic differentiation and bone nodule formation, together with reduced expression of osteoblast marker genes. Interestingly, the expression of OPG, an inhibitor of osteoclast differentiation was decreased in $Wnt1^{+/-}$ calvarial cells suggesting that altered RANKL/OPG ratio could affect the osteoclast phenotype observed *in vivo* in $Wnt1^{-/-}_{Prrx1}$ mice. We conclude that mesenchymal cell-derived Wnt1 is an essential regulator of bone metabolism that promotes osteoblast function and inhibits osteoclast differentiation.

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OC2.2

Deletion of Dickkopf-1 in osteoblasts or osteocytes increases bone volume in female mice

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Osteoporosis is a frequent disease leading to an increased risk of fractures caused by a systemic impairment of bone mass, strength, and microarchitecture. Given the emerging role of the Wnt signaling pathway in bone biology, we focused on the function of the important Wnt inhibitor dickkopf-1 (Dkk-1) and examined how the deletion of Dkk-1 solely in osteoblasts or osteocytes influences bone homeostasis. Therefore, we used the Cre-LoxP recombination system and crossed Dkk-1-floxed mice with osterix-cre (Osx; osteoblast-specific) and dentin matrix protein1-cre (Dmp-1; osteocyte-specific) mice. Female and male mice were examined at 10–12 weeks of age. However, the male mice did not show significant differences. Female mice without Dkk-1 in their osteoblasts showed a 3.75-fold increase in bone volume/total volume (BV/TV) compared to cre-negative controls. Furthermore, the trabecular number was increased by 59%, while the trabecular separation decreased by 38%. Cortical thickness and density were not changed. The deletion of Dkk-1 in osteoblasts seemed to have a significant impact on the amount of Dkk-1 in the serum as $Dkk\text{-}1^{OsxCre}$ mice had markedly lower Dkk-1 serum levels (−77%) compared to controls, whereas Dkk-1 serum levels were not changed in $Dkk\text{-}1^{Dmp1Cre}$ mice. However, $Dkk\text{-}1^{Dmp1Cre}$ mice also showed a significant increase in the BV/TV (twofold), the trabecular number (+33%), and the cortical thickness (+9%) while the separation was reduced (−26%). Histomorphometric parameters underlined these results as the mineral apposition rate and the bone formation rate were increased in both mouse lines. In summary, we show that most systemic Dkk-1 stems from osteoblasts and not from osteocytes. Nevertheless, the amount of Dkk-1 in osteoblasts and osteocytes is sufficient to modulate bone mass. Thus, the individual contribution of each cell type to the pathogenesis of specific bone diseases such as post-menopausal osteoporosis should be further investigated to potentially specifically target Dkk-1.

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OC2.3

Life-course GWAS approach for total body BMD unveils 16 new BMD loci with some exerting age-specific effects

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Introduction
Bone mineral density (BMD) is a highly heritable trait used to assess skeletal health in children and risk of osteoporosis later in life. To date >60 loci associated with bone-related traits measured at different skeletal sites have been identified. We conducted a genome-wide association study (GWAS) meta-analysis of total body (TB-)BMD in children and adults to identify genetic determinants and age-specific effects of loci on this trait.
Methods
We included 26 different study populations comprising ∼52 000 individuals with DXA measurements at different age ranges (0–15 years, $n=11\,800$; 15–45 years, $n=10\,600$; and >45 years, $n=30\,500$) and genetic data imputed to the *1000 Genomes* reference panel. Inverse variance meta-analysis was performed on TB-BMD adjusted for sex, age, weight, height and population stratification, for all the data and within each age strata. Genome-wide significance (GWS) was set at $P<5\times10^{-8}$. We compared effect sizes of leading variants between the two extreme groups.
Results
We identified GWS variants in 45 loci of which 16 are novel. Of these, 7 novel signals map in close vicinity of genes with a proven role in bone metabolism, *EN1*, *AQP1, RIC8A, CSF1, SLC8A1-AS1, MAFB* and *SMAD3*. Additionally, we identified loci with known skeletal specificity (*SOX6, LIN7C, RIN3, ABCF2*), age heterogeneity (*CPED1, C17orf53) and* bone compartment specificity (trabecular volumetric BMD, *FMN2;* or heel ultrasound, *TMEM135*). The strongest age-specific effects were found for variants in *ESR1* with GWS effect ($\beta=0.07$ s.d., $P=9.3\times10^{-13}$) only in adults ($P_{het}=3\times10^{-12}$) and *RIN3* with GWS effect ($\beta=0.1$ s.d., $P=1\times10^{-8}$) only in children ($P_{het}=7\times10^{-10}$).
Conclusion
TB-BMD is a relevant trait for genetic studies of osteoporosis, capable of identifying (novel) variants influencing different bone compartments at different skeletal sites. Applying an age-stratified GWAS approach allowed us to identify loci exerting effects at different stages of the lifespan, helping to unveil further the complex genetic architecture of osteoporosis.

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OC2.4

Up-regulation of Wnt antagonists contributes to the attenuated response of bone formation to repeat doses of sclerostin antibody in a mouse model

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Loss of the gene encoding the secreted Wnt antagonist sclerostin results in increased bone mass in humans and mice. Administration of antibodies to

sclerostin (Scl-Ab) has been shown to increase bone mineral density (BMD) by increasing bone formation and decreasing bone resorption, in both animal studies and human clinical trials. In these studies, the magnitude and rate of increase in bone formation markers, and the rate of increase of BMD, diminishes upon repeat dosing with Scl-Ab despite a continuous and progressive increase in BMD.

In this study, we investigated whether increased expression of secreted antagonists of Wnt signalling could be a contributory factor to the apparent attenuated response in markers of bone formation following repeat dosing of Scl-Ab.

Treatment of female Balb/c mice (8–10 weeks old) with Scl-Ab for five weeks (10 mg/kg weekly, s.c.) increased DXA whole body BMD by 9.4% ± 2.0% compared with vehicle control. Serum P1NP, a bone formation marker, was measured 4 days after one, three or five doses of Scl-Ab. After the first dose of Scl-Ab, serum P1NP significantly increased versus vehicle control (165% ± 9%) whilst the increase was less pronounced after the third or fifth dose of Scl-Ab (56% ± 11% or 21% ± 6%, respectively).

In order to investigate the mechanism of this attenuated response, mRNA expression of several secreted Wnt antagonists was determined in femurs collected from mice following five weeks of Scl-Ab or vehicle treatment. Expression of *SOST*, *SOST-DC1, DKK1, DKK2, SFRP2, FRZB, SFRP4, SFRP5* and *WIF1* transcripts was significantly increased (approximately 2–3.5 fold) upon Scl-Ab treatment compared with vehicle controls.

Continuous administration of Scl-Ab is associated with up-regulation of several Wnt antagonists. This could represent a negative feedback to increased Wnt signalling induced by Scl-Ab and may help explain the attenuation in the bone formation response and in bone density increase over time.

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OC2.5

Is circulating sclerostin an endocrine modulator of bone mass?

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Mechanosenstitive osteocytes in bone supress the local production of sclerostin in response to mechanical loading, to increase osteoblast differentiation and bone mass. In addition, sclerostin is secreted from osteocytes into the circulation. Serum sclerostin has been shown to correlate with osteoporosis and low bone mass, however there is limited evidence by which to determine whether serum sclerostin is acting either a biomarker or rather functionally to regulate bone mass. Our research attempted to address this in a conditional mouse model, producing localised sclerostin knockout, and reduced circulating sclerostin levels.

Limb-specific sclerostin null mice (*Prrx1-Cre Sost$^{f/f}$*) were generated, which retained sclerostin expression in the axial skeleton. These were compared to wild type mice and constitutive sclerostin null mice (*Sost$^{-/-}$*). The whole body dual-energy X-ray absorptiometry (DXA) was performed longitudinally to measure bone mineral content (BMC) and bone mineral density (BMD), as well as determination of lumbar and hind limb-specific measures. The three genotypes of mice were culled at 16 wk age to collect blood, femurs, and spine for further analyses.

We found that the serum sclerostin was reduced by 1.7-fold in *Prrx1-Cre Sost$^{f/f}$* compared to control mice, but was undetectable in *Sost$^{-/-}$* mice. The DXA results showed that greater BMD and BMC were present only in the hind limbs (limb BMD mean(SE) g/cm^2, control 0.05 (0.0007) Prrx1 0.069 (0.002), $P < 0.0002$) but not in the lumbar spine of *Prrx1-Cre Sost$^{f/f}$* mice (spine BMD, control 0.051 (0.0004) Prrx1 0.050 (0.0007), ns) whereas *Sost$^{-/-}$* mice showed greater BMD and BMC both in the limb and spine. Micro-computed tomography showed greater cortical bone mass in femurs of *Prrx1-Cre Sost$^{f/f}$* and *Sost$^{-/-}$* mice compared to control mice. Importantly, cancellous bone mass in vertebra did not differ between *Prrx1-Cre Sost$^{f/f}$* and control mice, despite the significant reduction in serum sclerostin in *Prrx1-Cre Sost$^{f/f}$*.

In conclusion, our results indicate that the local production of sclerostin is the primary factor in the modulation of skeletal bone density.

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OC2.6

N-cadherin maintains osteoprogenitor number and restrains Wnt signaling in osteoblasts

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We have shown that genetic ablation of *Cdh2* (N−cadherin gene) in osteolineage cells results in osteopenia and decreased osteoprogenitor number. Paradoxically, others have shown that mice overexpressing *Cdh2* in osteoblasts are also osteopenic; an action linked to a negative effect of N−cadherin (Ncad) on Wnt signaling, via sequestration of low density lipoprotein receptor−related protein−5 or 6 (Lrp5/6) and Axin. We hypothesize that Ncad has different effects on osteolineage cells depending on their differentiation stage: it supports mesenchymal and progenitor stem cells (MSPC), but restrains mature osteoblast activity. Conditional *Cdh2* ablation in Osx+ cells, which targets perinatal MSPC and osteoblasts, confirmed low trabecular bone mass (BV/TV by μCT), but also revealed significantly reduced body weight and size at age 2 months (20−25% and 15−20% *vs* wild type, respectively, $P < 0.05$). However, delaying *Cdh2* ablation until P28 (when Osx1 marks committed osteogenic cells but not MSPC) by doxycycline suppression of the Tet−sensitive *Osx1−Cre* transgene prevented the osteopenia and growth defect. In fact, trabecularization of the diaphyses was significantly larger in post−natally *Cdh2* ablated mice ($P < 0.05$), consistent with a negative action of Ncad in more mature osteogenic cells. Supporting the hypothesis that Ncad maintains MSPC, bone mass and osteoprogenitor number were also reduced upon early Cdh2 ablation by Prx1−Cre. Conversely, introduction of one Dkk1−resistant Lrp5 mutation (Lrp5^{A214V}) associated with high bone mass, in cKO^{Osx1} background completely rescued the osteopenia but not the early growth defect. Indeed, cKO^{Osx1}; Lrp5^{A214V} compound mutant mice in the first month of life were 20−25% lower in weight than Lrp5^{A214V} mice, but as adults displayed high bone mass (BV/TV by μCT). Thus, Ncad is involved in MSPC maintenance and early post-natal skeletal growth; the latter action is independent of Ncad restraining effect on Wnt signaling. Interference with Ncad in adults may result on bone anabolism without potential detrimental effects on osteoprogenitors.

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Clinical trials, FGF-23 and focal osteoporosis

OC3.1

Effect of odanacatib on bone density and estimated bone strength in postmenopausal women: a CT-based sub-study of the phase 3 long-term odanacatib fracture trial (LOFT)

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Odanacatib (ODN), a selective oral inhibitor of cathepsin K, is in development for the treatment of osteoporosis. In the Phase 3, Long-Term ODN Fracture Trial (LOFT; NCT00529373), ODN significantly reduced fracture risk. This imaging sub-study primarily investigated the effect of ODN on volumetric BMD (vBMD) of the lumbar spine (LS) and total hip (TH) using quantitative computed tomography (QCT).

Women aged ≥65 without baseline radiographic vertebral fracture (VFx) and TH or femoral neck (FN) BMD T-score < −2.5, or with prior VFx and TH or FN T-score < −1.5 and TH and FN T-score > −4.0, were randomised (1:1) to ODN 50 mg/week or placebo. All received vitamin D_3 (5600 IU/week), and calcium. Endpoints included % change from baseline in LS trabecular vBMD (primary) and TH cortical vBMD (secondary) at 24 months. Additional QCT endpoints included % change from baseline in LS and TH trabecular, cortical and integral vBMD, integral TH BMC, and estimated whole-bone strength by finite element analysis (FEA) at 24 months.

In this sub-study, 164 women (78 ODN, 86 placebo) were enrolled. Treatment with ODN increased vBMD and BMC versus placebo at all sites, including LS trabecular vBMD (treatment difference [95% CI] 8.9 [3.9, 13.9]; $P < 0.001$), TH cortical vBMD (2.8 [1.4, 4.1]; $P < 0.001$), integral vBMD at LS (8.5 [5.7, 11.4]; $P < 0.001$) and TH (5.3 [3.7, 7.0]; $P < 0.001$), and TH integral BMC (5.1 [3.3, 6.8]) at 24 months. ODN numerically increased whole-bone estimated strength versus placebo at the L1 vertebra (mean % change from baseline [95% CI] 9.0 [6.0, 12.0] *vs* −0.8 [−4.2, 2.6]) and proximal femur (3.8 [2.3, 5.3] *vs* −3.1 [−4.4, −1.8]) at 24 months.

In postmenopausal women with osteoporosis, ODN compared with placebo increased trabecular, cortical and integral vBMD in the LS and TH, integral TH BMC, and whole-bone estimated strength at the spine and hip.

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OC3.2

Effects of Denosumab (Dmab) on bone matrix mineralization: results from the phase 3 FREEDOM trial

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Low fracture (FX) incidence has been demonstrated in women with postmenopausal osteoporosis (PMO) treated with DMAb for up to 10 years in the FREEDOM extension [Bone ASBMR 2015]. Bone biopsy-based assessment of DMAb's effects at the tissue level has demonstrated a low remodelling rate consistent with DMAb's mechanism of action (Reid *JBMR* 2010; Brown *JBMR* 2014). From FREEDOM, we report the effects of DMAb on bone matrix mineralization in women who underwent transiliac crest bone biopsy at year 2 and/or 3 (Reid *JBMR* 2010). Bone matrix mineralization was assessed in a blinded fashion by digitized quantitative microradiography and analyzed using a Matlab program (Montagner *J X-Ray Sci Technol* 2015). The mean degree of mineralization of bone (DMB) and heterogeneity index (HI) of the distribution of DMB were calculated for cancellous and cortical bone, endocortical and periosteal sub-compartments of cortical bone, and total bone. 72 of 115 biopsies (42 DMAb, 30 Placebo) from the FREEDOM bone biopsy sub-study were evaluated. Subject demographics were comparable to FREEDOM. DMAb resulted in a significant increase in mean DMB compared with Placebo (Table 1; findings consistent across cancellous and cortical compartments). A significantly lower HI was observed in total bone and in all compartments assessed in the DMAb-treated group ($P<0.05$), consistent with reduced bone turnover in response to DMAb. In women with PMO, DMAb resulted in increased bone matrix mineralization and a lower HI compared with Placebo. These data are consistent with expected results based on observations with other antiresorptives (Bala *Eur J Endocrinol* 2011) and with DMAb's mechanism of action.

Table 1 Mean (SD) DMB by Location

	DMAb	Placebo	*P*-value*
Cancellous bone	1.036 (0.035) [*n*=41]	1.009 (0.034) [*n*=30]	0.0014
Cortical bone	1.100 (1.066) [*n*=42]	1.066 (0.032) [*n*=30]	0.0002
Endosteal	1.106 (0.041) [*n*=31]	1.074 (0.032) [*n*=22]	0.0017
Periosteal	1.095 (0.043) [*n*=31]	1.067 (0.036) [*n*=22]	0.0085
Total bone	1.079 (0.035) [*n*=42]	1.053 (0.030) [*n*=30]	0.0009

*P-value of 2-sided Wilcoxon test for between-group comparison in DMB

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OC3.3

Vitamin D supplementation reduces pregnancy chances: a randomized, placebo-controlled trial

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Introduction
Low 25-hydroxyvitamin D (25OHD) levels are frequently found in fertile women and associated with low birth weight, reduced fertility and adverse pregnancy outcomes.
Objective
We investigated the effects of vitamin D supplementation on birth weight, fertility, and delivery complications in women planning pregnancy with low 25OHD levels.
Methods
This was an investigator-initiated double-blind, randomized, placebo-controlled, parallel-group trial from a single centre.
Patients
A total of 193 women planning pregnancy (20–40 y old) with 25OHD level below 50 nmol/l were recruited.
Interventions
Daily supplementation of cholecalciferol 70 μg (2800 IU) (70-VitD_3), 35 μg (1400 IU) (35-VitD_3) or matching placebo was administered before conceiving and continued until 16 weeks post partum.
Main outcome measures
25OHD, birth weight, fertility, and complication were evaluated.
Results
Baseline level of 25OHD did not differ between groups (mean 43 nmol/l; $P=0.91$). Levels of 25OHD increased dose-dependently in response to treatment ($P<0.001$). A total of 108 women (56%) conceived within 12 months after randomization, 41 (38%) in the placebo group, 31 (29%) in the 70-VitD3, and 36 (33%) in the 35-VitD3 group. Compared with placebo, the 35-VitD3 group did not affect pregnancy chances significantly (HR: 0.79; 95% CI: 0.48, 1.28), but chances of conceiving were significantly reduced by a daily supplement of 70 μg (HR: 0.52; 95% CI: 0.31, 0.87). Birth weight did not differ significantly between the vitamin D3 treated and the placebo group. Complications during labour were significantly more frequent in the placebo- compared with the combined vitamin D3 group (52 *vs* 23%, $P<0.005$), although specific complications did not differ between groups. There were no differences between groups on any safety measures.
Conclusions
High doses of vitamin D_3 (70 μg/daily) may reduce the likelihood of conceiving, but may also be associated with fewer complications during childbirth without affecting birth weight.
Approved by The Ethical Committee of Denmark (M-20090097).

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OC3.4

The response of fibroblast growth factor-23 to teriparatide in postmenopausal osteoporosis

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

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OC3.5

Low serum iron is associated with high serum FGF23 in elderly men: the Swedish MrOS study

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Introduction
Fibroblast growth factor (FGF23) is a hormone derived from osteoblasts and osteocytes being involved in calcium and phosphate homeostasis, where serum iron (S-Fe) has been suggested as a potential mediator of FGF23 regulation. The aim was to determine whether iron status is a determinant of FGF23 in elderly men.
Methods
The MrOS (osteoporotic fractures in men is a population based study of elderly men, in the Gothenburg part, (median age of 75.3, range 70.5–81.0 years) 955 men without ongoing medication or supplementation with iron were included. Serum intact FGF23 was analyzed with a two-site monoclonal antibody based ELISA (Kainos Laboratories International; Tokyo, Japan). Baseline data included serum levels of intact FGF23, S-Fe, transferrin saturation (TS) and ferritin before and after adjusting for potential confounders.
Results
Log FGF23 correlated (age adjusted) negatively with S-Fe ($r=-0.15$, $P<0.001$), TS ($r=-0.16$, $P<0.001$), and ferritin ($r=-0.07$, $P=0.03$). There was a significant difference in mean S-Fe between quartiles 1 and 3 of FGF23 compared with quartile 4 (20.0 μmol/l vs 18.5 μmol/l, $P<0.001$), still significant after further adjustment for age, BMI, Cystatin C, hs-CRP, 25 hydroxyl vitamin D, phosphate and PTH ($P=0.045$). Subjects with TS < 15% (3.2%, 31/955) had higher mean FGF23 compared with subjects with TS ≥ 15% (59.0 μmol/l vs 46.1 μmol/l, $P=0.008$), still significant after adjustment for age (P log = 0.007).
Multiple stepwise linear regression analyses, (adjusted for age, BMI, smoking, cystatin C, hs-CRP, 25 hydroxyl vitamin D, phosphate, calcium, PTH, erythropoietin, hemoglobin, total cholesterol, were performed in three separate models. S-Fe and TS were then independent predictors of FGF23 (standardized β −0.11, $P<0.001$ and −0.10, $P<0.001$ respectively) whereas ferritin was not.
Conclusions
Low S-Fe is in old men associated with high levels of intact FGF23, independently of markers for inflammation and renal function, suggesting an iron related pathway in FGF23 regulation.

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OC3.6

Focal osteoporosis associated with hip fracture involves both trabecular and cortical bone; a 3D cortical bone mapping study of cases and controls using clinical CT

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Focal cortical thinning and loss of trabecular structure in the proximal femur is associated with hip fracture. We analysed clinical computed tomography (CT) scans in cases and controls to explore their contributions to hip fracture in women. We used cortical bone mapping (CBM) and statistical parametric mapping (SPM) after combining women with hip fracture from FEMCO and Prague Hip Joint in Trauma studies ($n=138$, 52 Trochanteric and 86 Femoral Neck) and comparing their bone parameters with 121 healthy age-matched female controls from previous Cambridge and Prague studies. Our aim was to determine how well focal measures of Cortical (Mass Surface Density, CMSD) and Trabecular bone (Endocortical trabecular density, ECTD), compared with areal BMD (aBMD, DXA-like from CT) in discriminating hip fractures from controls using ROC analysis. We aligned participant selection and CT scanning criteria across centres, and also modelled study site within the general linear model. An average single measure of 3D CMSD or ECTD was taken for each patient from previously determined bone mapping ROIs. We tested the ability of age, height and average measures of either aBMD, CMSD, ECTD or combined CMSD + ECTD to correctly discriminate fractures. The corresponding AUC values and 95% CI's were calculated (Table 1).

Table 1

	aBMD AUC (95% CI)	CMSD AUC (95% CI)	ECTD AUC (95% CI)	CMSD + ECTD AUC (95% CI)
All hip Fx	0.79 (0.73–0.85)	0.73 (0.67–0.79)	0.83 (0.77–0.88)	0.82 (0.76–0.87)
Neck Fx	0.77 (0.70–0.83)	0.79 (0.72–0.84)	0.81 (0.73–0.85)	0.84 (0.77–0.88)
Troch Fx	0.74 (0.66–0.80)	0.70 (0.60–0.77)	0.80 (0.73–0.85)	0.82 (0.75–0.87)

Femoral neck and trochanteric fracture patients had different patterns of focal osteoporosis. As reflected in ROC analysis and odds ratios, the ability to effectively discriminate hip fractures from controls was dependent upon including both trabecular and cortical measurements. AUC for discrimination of hip fracture type was 0.794 for areal BMD, and 0.822 for 3D measures. These 3D CBM measures warrant testing in a prospective female cohort.

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Catabolism and metabolism

OC4.1

A small molecule inhibitor of TRAF6 dependent signaling reduces osteoclastogenesis and prevents ovariectomy induced bone loss

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Tumour necrosis factor receptor-associated factor 6 (TRAF6) plays a key role in osteoclastogenesis through the regulation of RANK/CD40 TRAF6-mediated signaling. Mice deficient in TRAF6 exhibit high bone mass and were protected against inflammation-induced bone loss. Here we describe the effects of a small-molecule 6877002 that has been shown to prevent the binding of TRAF6 to its domain on RANK/CD40 receptor on osteoclast formation *in vitro* and *in vivo* and on ovariectomy-induced bone loss *in vivo*. The TRAF6 inhibitor 6877002 (1 μM) inhibited RANKL- and/or CD40L-induced osteoclast formation (86%, $P<0.001$) and survival (57%, $P<0.001$) without affecting pre-osteoclast viability. Moreover, this agent (1 μM) inhibited the ability of osteoblasts to induce osteoclast formation and activity in murine osteoblast-bone marrow co-cultures without affecting osteoblast viability. In human T cells, 6877002 (1 μM) completely prevented T cell-induced osteoclast formation (91%, $P<0.001$) and significantly reduced mature osteoclast survival (54%, $P<0.001$) without affecting T cell survival. Pretreatment of mature osteoclasts and their precursors generated in M-CSF and RANKL stimulated mouse bone marrow cultures or M-CSF dependent bone marrow macrophages with 6877002 (10 μM) for 60 min prior to stimulation with RANK-L (100 ng/ml) or CD40L (100 ng/ml), completely abolished the phosphorylation of IKKαβ and prevented NFκB activation. The TRAF6 inhibitor 6877002 (10 mg/kg per day) completely prevented the loss of trabecular bone (40% increase, $P<0.01$) following ovariectomy and preserved trabecular number and thickness ($P<0.05$), and increased trabecular connectivity in mice. Histomorphometric analysis showed that this was mainly due to a reduction in osteoclast number and activity ($P<0.05$) with no significant effect on osteoblast number. Altogether, these findings suggest that the small molecule TRAF6 inhibitor 6877002 and related compounds have great potential as a novel class of anti-resorptive agents, which may be of clinical value in the treatment of diseases characterized by excessive osteoclastic bone resorption such as postmenopausal osteoporosis, rheumatoid arthritis and cancer-associated bone disease.

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OC4.2

Inhibition of Sphingosine 1 Phosphate produced by Osteoclasts reduces chondrocyte catabolism and prevents osteoarthritis in mice

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Purpose
High osteoclastogenesis accompanies early stages of osteoarthritis (OA). Cartilage loss is reduced when osteoclasts are inhibited in mice models with bone hyperresorption. Although several evidences show that osteoclast-produced molecules affects chondrocyte metabolism, the mechanism by which inhibition of osteoclasts protects from cartilage damage is unclear. Our purpose was to investigate the role of Sphingosine 1 Phosphate, a lipid mediator secreted by osteoclasts known as mediator of bone remodeling, in chondrocyte metabolism and OA.

Methods
Primary murine chondrocytes and cartilage explants were cultured in the presence of osteoclast conditioned media (Oc-CM) to quantify matrix protein expression and proteoglycan content (RT-qPCR, WB, safranin O staining). S1P receptors antagonists, JTE-013 for S1P receptor 2 (S1PR2) and VPC 23019 for S1PR1-3 as well as siRNA strategies are used to block S1P signaling. The role of S1P signaling in OA was investigated in the DMM model by JTE-013.
Results
Femoral head explants and primary murine chondrocytes cultured in presence of Oc-CM induced lower extracellular matrix production (proteoglycan release) and higher metalloprotease expression (MMP3, MMP13, ADAMTS-4, ADAMTS-5) compared to controls. The expression of S1P receptors 1-3 was increased in chondrocytes cultured with Oc-CM, as well as the activation of their signaling pathway (MAPK). However, only the inhibition of the receptor S1PR2 by JTE-013 and RNA silencing abolished Oc-CM effect on MMP-3 and -13 in primary chondrocytes, but not those of S1PR1-R3. S1PR2 inhibition was confirmed in femoral head explants as JTE-013 reduced loss of proteoglycan and extracellular matrix degradation initially induced by Oc-CM. In OA mice, systemic administration of JTE-013 reduced OA score (5.5 ± 0.86 vs 4 ± 0.70)
Conclusion
Osteoclast-secreted factors disrupt the balance of chondrocyte metabolism. The activation of S1P signaling in chondrocytes by osteoclasts promotes chondrocyte catabolism, the inhibition of which prevented OA. Therefore, subchondral bone manipulations may affect chondrocyte function and OA.

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OC4.3

Connecting the dots between bone and energy metabolism: the role of Lipocalin 2

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We previously demonstrated the involvement of Lcn2 in bone loss induced by mechanical unloading. This prompted us to investigate bone phenotype of $Lcn2^{-/-}$ mice by μCT, which showed an osteopenic phenotype, characterized by 40% lower trabecular bone volume, 50 and 21% lesser trabecular number and thickness, respectively, and 20% higher trabecular separation, compared to WT, while cortical thickness was significantly lower (40%) only in elderly $Lcn2^{-/-}$ mice. $Lcn2^{-/-}$ mice showed 30% and 50% lower osteoblast number and surface, and 50% lower bone formation rate, while osteoclast parameters were unremarkable. Consistently, femurs transcriptional levels of Alp, Runx2 and Col1A2 were significantly lower in $Lcn2^{-/-}$ mice. We found no difference of ALP activity and nodule mineralization in calvaria osteoblast cultures from WT and $Lcn2^{-/-}$ mice, while less ALP positive colonies were observed in bone marrow-derived $Lcn2^{-/-}$ cells. Incidentally, we noticed that $Lcn2^{-/-}$ mice showed higher body weight at all ages evaluated, which prompted us to investigate their energy metabolism. We observed lower serum levels of fasted glucose (60.58 ± 7.48 vs 83.10 ± 18.9 mg/dl, $P=0.008$), likely due to higher circulating insulin (3.67 ± 0.7 vs 2.25 ± 0.79 ng/ml, $P=0.036$). Consistently, glucose tolerance was significantly higher in $Lcn2^{-/-}$ compared to WT mice. Interestingly, while insulin tolerance test was similar at 3 months of age, 12-month-old $Lcn2^{-/-}$ mice showed 20% lower insulin sensitivity. Finally, the transcriptional expression of the insulin receptor was 30% lower in $LCN2^{-/-}$ osteoblasts compared to WT, which could explain why, despite the increased levels of insulin, known to be a bone anabolic hormone, $Lcn2^{-/-}$ mice showed an osteopenic phenotype. This hypothesis was also supported by the observation that the insulin receptor downstream gene, osteocalcin, was significantly reduced in $Lcn2^{-/-}$ mice ($P=0.001$). Taken together, these results point to Lcn2 as a key player of the crosstalk between bone and energy metabolism, contributing to the insulin pathway.

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OC4.4

Osteocyte-specific ablation of Pparγ improves energy metabolism and prevents fat accumulation but not bone loss in response to a high fat diet

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Pparγ is a master transcriptional regulator of energy metabolism. We demonstrated that Dmp1-Cre/Lox-mice (KO) have increased bone mass and improved energy metabolism. Here we investigated if Pparγ-deficiency in Dmp1 cells can prevent high fat diet effects on these parameters. For this purpose, WT and KO male mice aged of 16 weeks received a high fat or chow diet (HF 60% vs CD 10% of fat) for 12 weeks. Lean and fat, bone structure, metabolic rate and tissue temperature were evaluated respectively by echoMRI, microCT, labmaster and infrared camera.
As expected vertebral BV/TV was higher in KO (+39% vs WT, $P<0.01$) and lower in HF (−12% vs CD) mainly due to an effect on thickness (−17% vs CD, $P<0.01$) but there was no interaction between diet and genotype. Cortical structure was not affected by diet. Under HF, movement, VO_2 and heat were higher in KO (+41%, +13%, +13% vs WT, $P<0.05$). Body temperature was also higher, particularly in the BAT-neck region (+1.5% vs WT, $P<0.01$). UCP1&3 and PPARβ&γ expression in BAT was higher in KO (84, 139, 125 and 167% vs WT, $P<0.01$). Histology and UCP1 expression indicate a browning of the WAT. As a result, glucose and insulin tolerance test were improved in the KO (AUC −22 and −9% vs WT, $P<0.05$). Finally, HF induced fat mass increase was prevented in KO (+17% vs CD and +44% vs CD in WT, $P<0.05$) whereas increased in lean mass was greater (+14.2% vs CD and +9.6% vs CD in WT). In conclusion, ablation of Pparγ by Dmp1-Cre improves bone mass but does not prevent the deleterious effects of HF on bone. In contrast, it improves BAT activity and insulin sensitivity, preventing fat accumulation and improving glucose homeostasis. How bone regulate energy metabolism under the control of Pparγ remains to be determined.

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OC4.5

Effects of glucagon-like peptide-1 receptor agonists on bone blow flow and architecture in diabetic mice

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Type 2 diabetes mellitus prevalence increases with age and the disease predisposes to increased risk of fractures and skeletal complications. One possible contributor to skeletal weakening in diabetes is a decline in blood supply. We hypothesised that bone blood flow is impaired in diabetic mice and that chronic administration of glucagon-like peptide-1 receptor agonists (GLP-1RA) can increase blood flow to bone, thereby stimulating bone formation and improving bone architecture.
Nine weeks old male diabetic (db/db) and control mice were daily injected subcutaneously for 28 days with saline or the GLP-1RA Exenatide (Ex-4) (10 μg/kg per day) ($n=10$/group). The effect of Ex-4 on hind limb perfusion was measured by laser Doppler imaging. Tibial bone architecture was imaged by micro-CT *ex-vivo*.
Diabetic mice had −40% lower bone blood flow than control mice ($P<0.0001$) at baseline. Ex-4 acutely increased tibial blood flow in diabetic mice from 15 min of injection to a maximum of 25% increase compared to saline ($P<0.0001$). Similarly, blood flow was increased with Ex-4 in control mice but at a lower extend than in diabetic mice (+20%, $P<0.05$). No chronic effect of Ex-4 was shown when blood flow was monitored after the last injection.
Diabetic mice have lower trabecular bone mass compared to controls, due to decreases in trabecular number and thickness. They also exhibit impaired bone connectivity, structure and cortical bone geometry. Ex-4 treatment increased trabecular bone volume (+49%, $P<0.01$), thickness (+8%, $P<0.01$) and number (+38%, $P<0.01$) in diabetic but not in control mice. Connectivity and structure were also improved as shown by decreased trabecular pattern factor (−29%, $P<0.0001$) and structure model index (−11%, $P<0.01$).
In conclusion, our results suggest that diabetic mice have lower blood flow and impaired skeletal structure and that Ex-4 exert a bone anabolic action in diabetic mice that could be in part due to its increased skeletal perfusion.

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OC4.6

The myokine Irisin improves bone quality and strength

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Irisin is a hormone-like myokine secreted from skeletal muscle in response to exercise. Considering that an intimate relationship between skeletal muscle and bone has been well established and that physical exercise physiologically stimulates the skeleton strengthening, we explored the involvement of Irisin on bone metabolism.
Our findings demonstrated that the myokine Irisin improves cortical bone mass and geometry *in vivo*, supporting the idea that Irisin recapitulates some of the most important benefits of physical exercise on the skeleton and plays protective role on bone health. Healthy young male mice, treated with a micro-dose of recombinant Irisin (r-Irisin), showed increased cortical bone mineral density (+7.15%; $P<0.01$), periosteal circumference (+7.5%; $P<0.03$) and polar moment of inertia (+19.21%; $P<0.01$). The enlarged bone perimeter and cross-sectional area, thus distributing bone mass further from the center of bone, indicated improved resistance of tibia from Irisin-treated mice to bending forces. Furthermore, the increase of polar moment of inertia, an index of long bone resistance to torsion, provided evidence that Irisin-treatment might maximize bone to become more structurally efficient for torsion, in order to induce optimal stress transfer and physical performance. Likewise, three-point bending tests of tibiae showed that bending strength (+65%; $P<0.01$) and energy to fracture (+9.5%; $P<0.05$) increased in Irisin-treated mice. Dynamic histomorphometry of the tibial cortical bone, using timed injections of xylelol orange and calcein, showed a significant increase in bone formation rate (+85%; $P<0.01$) and mineral apposition rate (+70%; $P<0.01$).
This new revealed role for Irisin as mediator of bone-muscle connection has better clarified the molecular mechanisms underlying the positive effect of physical exercise on bone. Future extension of our findings could support the design of an Irisin-based therapeutic strategy that may be relevant for the treatment and prevention of osteoporosis during aging, immobility, muscle wasting (sarcopenia) and absence of mechanical load (microgravity).

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Risk factors for fracture, Pagets disease of bone and musle and bone

OC5.1

High serum miRNA 550a-5p levels are risk factors for incident fractures in older postmenopausal women with type 2 diabetes

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Standard DXA measurements, including FRAX scores, are limited in assessing fracture risk in type 2 diabetes (T2D). Novel, general applicable biomarkers are therefore needed. MicroRNAs (miRNAs) are secreted into the circulation from cells of various tissues proportional to local disease severity. Serum miRNA classifiers including miR-550a-5p were recently found to discriminate T2D women without and with prevalent fragility fractures with high specificity and sensitivity (AUC > 0.90). However, the role of miR-550a-5p in predicting diabetic fragility fractures and its effect on osteogenic and adipogenic differentiation is unknown.
The AGES-Reykjavik cohort encompasses 330 T2D postmenopausal women. After excluding all subjects with bone-affecting medications/diseases, 171 T2D women remained for analysis. Of those, 71 experienced an incidence skeletal fracture during the 10-year follow-up. Baseline serum miR-550a-5p levels were determined by qPCR-arrays in all 171 T2D postmenopausal women. Decision tree models – using age and miRNA expression values as decision points – were employed to identify T2D patients at highest risk for developing incident skeletal fractures. MiR-550a-5p was further assessed by overexpression and knockdown in an in-vitro model of osteogenic differentiation and for its effect on adipogenic differentiation.
We found that T2D postmenopausal women ≥76 years had a risk of 66.7% to develop an incident skeletal fracture over the next 10 years if they had a high serum concentration of miR–550a-5p (qPCR Ct-values of <39). In contrast, T2D women ≥76 years with low serum miR-550a-5p levels (qPCR Ct-values of ≥39) had only a 30% risk of developing an incident fracture, as did subjects <76 years (23% risk). *In-vitro* results confirmed that miR-550a-5p inhibits osteogenic differentiation and matrix mineralization and impairs adipogenic differentiation. Overall, these data provide first proof that biochemical markers such as serum microRNAs can be used to predict fracture risk and to identify high-risk T2D fracture groups that may benefit most from treatment.

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OC5.2

Impaired bone material properties increase the risk of all fractures and severity of vertebral fractures in osteoporosis: an impact microindentation study

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Context
Previous impact microindentation studies have demonstrated that osteoporosis patients exhibit impaired bone material strength, which may contribute to skeletal fragility. Whether the impact differs between subtypes of fractures has, however, not been evaluated yet.
Objective
To test whether impaired bone material properties differ between different subtypes of osteoporotic fracture and assess how this property relates to fracture severity.
Methods
We measured bone material strength index (BMSi) by impact microindentation in 60 normal controls and 74 women with osteoporosis with or without fractures. Bone mineral density by DXA and markers of bone turnover were also assessed. Vertebral fracture severity was determined by semi-quantitative (Q3) grading of lateral X-rays of the spine from the DXA-scanner.
Results
BMSi was found to be significant lower in subjects with osteoporotic fractures than in controls (77±7.1 vs 71.2±6.5 $P<0.001$). Moreover, a significant negative correlation was observed for BMSi on fracture Q3 severity, which remained significant after adjusting for age and total hip BMD ($r^2=0.19$, $P=0.008$). Each incremental decrease of one standard deviation in BMSi was associated with a 4.5-fold increased risk of fracture, after adjustment for age, weight and height (OR 4.45; 95% CI 1.07, 19.21 $P=0.04$). The receiver operator curve (ROC) area under the curve (AUC) for BMSi for patients with vertebral fracture, hip fracture and non-vertebral non-hip fracture were 0.758 (0.648–0.868); 0.712 (0.576–0.848) and 0.668 (0.527–0.809), respectively.
Conclusion
Impaired bone material properties constitute an independent risk factor for all important osteoporotic fractures (vertebrae, hip, non-vert-non-hip) and is also related to the severity of vertebral fractures.

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OC5.3

Osteoprotegerin autoantibodies are independently associated with low hip bone mineral density and increased fracture risk in patients with ankylosing spondylitis

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Osteoporosis and vertebral fractures are recognised complications of ankylosing spondylitis (AS) but the underlying causes are incompletely understood. Osteoprotegerin (OPG) is a decoy receptor for RANK-L and inhibits osteoclastogenesis. We have previously demonstrated that antibodies to OPG (OPG-Ab) are associated with osteoporosis and increased bone turnover in patients with autoimmune diseases. The aim of this study was to determine whether OPG-Abs were detectable in AS patients and relate these to bone health. Patients with AS were recruited from outpatient clinics at two centres in the UK between 2011 and 2015. Patient demographics, disease characteristics and fracture history were recorded. BMD was assessed by antero-posterior DEXA. Serum levels of OPG-Ab were measured using an in-house ELISA and considered to be positive if values were above three S.D.s above mean in healthy controls. Associations between OPG-Ab and BMD and fractures were assessed using logistic regression, adjusted for age, gender, duration since diagnosis, BMI and study centre.

We studied 134 patients, of whom 75% were male. The mean age was 47 (S.D. ±15) years and median disease duration 6.5 years. 16 patients were tested positive for OPG-Ab (11.9%). The presence of OPG-Ab was associated with lower hip BMD ($P=0.018$), and an increased number of fractures ($P=0.007$). There was no association between OPG-Ab and patient demographics, disease characteristics or activity. Logistic regression revealed an association between OPG-Ab and disease duration (OR 1.04; 95% CI 1.00, 1.07; $P=0.045$) and a strong independent association with hip T-score (OR_{adj} 0.43; 95% CI 0.22, 0.85; $P=0.015$) and history of fractures (OR_{adj} 4.78; 95% CI 1.37, 16.7; $P=0.014$).

In conclusion, this cross-sectional study demonstrates that OPG-Ab were present in 11.9% of AS patients. OPG-Ab was strongly and independently associated with hip BMD and history of fractures. This raises the possibility that OPG-Ab may play an important role in accelerated bone loss and increased fracture risk in AS.

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OC5.4

Fine mapping of the chromosome 1p13 locus for susceptibility to Paget's disease of bone

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Introduction

Paget's disease of bone (PDB) is a common disorder characterised by increased and disorganised bone remodelling. Previous genome wide association studies identified a locus for susceptibility to PDB on chromosome 1p13, tagged by rs484959 which lies 87 kb upstream of the CSF1 gene. This is a strong candidate for PDB since it encodes macrophage colony-stimulating factor (M-CSF) a critical cytokine for osteoclast formation and survival.

Purpose

To conduct bioinformatic analysis and fine mapping of the chromosome 1p13 susceptibility locus to identify the mechanisms by which genetic variants in this region predispose to PDB.

Methods

Bioinformatic analysis was conducted using the ENCODE database to identify potential regulatory motifs in the region of strongest association and we conducted fine mapping of the region using a combination of next-generation sequencing and Sanger sequencing in 272 PDB cases and 110 controls to identify potentially causal variants.

Results

Bioinformatic analysis showed that two of the three strongest hits on GWAS were within an H3K27Ac mark and close to DNAase1 hypersensitivity sites, both of which suggest that the mechanism of association might be through an effect on gene regulation Mutation screening of the coding regions of CSF1 gene identified three missense mutations but at equal frequency in cases and controls. Sequencing of the elements in a 5 kb region surrounding the top hit revealed variants within several potential regulatory elements that were greatly enriched in PDB cases versus controls. The most strong association was with a SNP within a region predicted to bind AP1 and CEBP and POLR2 site 46.7 vs 12%, $P=1.44\times10-10$) which have key roles in regulating bone turnover.

Conclusion(s)

These observations are consistent with the hypothesis that common genetic variants located upstream of CSF1 predispose to the PDB by modulatory effects on regulation of CSF1. Further studies are in progress using CRISPR/Cas to disrupt the region and investigate the effects of CSF1 mRNA expression in cell lines and to examine the relation between variants at the 1p13 locus and circulating levels of MCSF in patients with PDB and controls.

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OC5.5

The relationship between muscle strength and bone outcomes in ageing UK men

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Morbidity and mortality are associated with osteoporosis and sarcopenia. There are few data describing the associations between functional measures of muscle and bone. Therefore, the aim of this study was to examine the associations of muscle strength and tibial bone outcomes in ageing men.

Men ($n=301$) aged 40–85 years were recruited in UK (201-White, 43-Black, 57-South-Asian). pQCT was performed at the 38 and 66% tibia with the outcome measures: cortical bone mineral content (Ct.BMC), cross-sectional area (CSA), and cross-sectional moment of inertia (CSMI). Muscle strength was assessed by jumping mechanography: a single 2-leg counter-jump to calculate muscle force (kN) and power (kW). Linear regression was used to explore the relationship between force and bone outcomes, adjusting for ethnicity, age, weight and height. Similar analyses were used to identify the effects of power and age, and included pairwise comparisons due to finding ethnicity-age interactions. Results are expressed as β-coefficients (95% CI) of percentage unit change in force/age.

Muscle force was a significant predictor of bone. At the 38% site, for every 1-unit increase in force, there was an increase of 8.5% (3.6, 13.4) in Ct.BMC; 9.3% (5.4, 13.2) in CSA and 18.6% (11.1, 26.2) in CSMI (all $P<0.001$). Similar effects were seen at the 66% site. There were no effects of ethnicity in force predicting tibial bone outcomes. Muscle power significantly declined with age in all groups: Whites (−1.9%; −2.1, −1.7), Blacks (−1.3%; −1.8, −0.8), South-Asians (−1.8%; −2.3, −1.3). There was a trend for an interaction with ethnicity in the power and age relationship, with a significant difference between White and Black men ($P=0.03$).

Muscle force positively predicted bone outcomes in ageing men. An absence of an ethnic effect suggests biomechanical adaptations are the main driver of this association. In contrast, the relationship between power and age was different between ethnicities suggesting a greater environmental influence which may contribute to falls.

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OC5.6

The metabolic alterations behind bone fragility in Duchenne muscular dystrophy

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Low bone mineral density (BMD) and an increased rate of both peripheral and vertebral fractures have been observed in patients with Duchenne muscular dystrophy (DMD), but studies on bone metabolic alterations in this disease are still very few.
We are now presenting the preliminary findings of an ongoing multicenter, prospective study aimed to identify the characteristics of DMD boys carrying a higher risk of bone loss and fractures, through the evaluation of BMD, bone turnover parameters, and genetic configuration.
On 37 DMD boys (mean age 10.6 ± 3.2 years), we evaluated BMD (by DXA), bone mineral apparent density (BMAD), bone turnover markers (plasma osteocalcin (OC); serum bone-specific alkaline phosphatase (BSAP) and C-terminal telopeptide (CTx); urinary N-terminal telopeptide (NTx)), serum osteoprotegerin (OPG), receptor activator of nuclear factor kappa-B ligand (RANKL) and (for the first time in DMD boys) serum Dickkopf related protein 1 (Dkk1).
At baseline evaluation, 28/37 patients (75.7%) had a significantly reduced lumbar spine BMAD (*Z*-score ≤ -2). OC was 50.42 ± 22.28 ng/ml; BSAP 23.13 ± 8.58 pg/ml; CTx 738.20 ± 349.26 ng/ml; NTx 333.92 ± 251.82 nMBCE/mMCR. While bone formation markers (OC, BSAP) were within normal range for age, bone resorption markers (CTx, NTx) were increased ($P < 0.05$). The RANKL/OPG ratio was significantly higher than normal (112.3 ± 107.2; normal controls 28 ± 11; $P < 0.001$), while Dkk1 was lower than normal (17.44 ± 17.1 pg/ml; normal controls 37 ± 18.3 pg/ml; $P < 0.02$). Significant correlations were observed between RANKL/OPG ratios and BMAD *Z*-scores ($P = 0.03$) and between Dkk1 levels and BMAD *Z*-scores ($P < 0.005$).
These data confirm that the BMD reduction observed in DMD seems due to increased bone resorption. Moreover, the imbalance between RANKL and OPG, and the insufficient compensation due to Dkk1 reduction could also concur in determining the low BMD and increased fragility fracture risk in boys affected by DMD, although further studies are needed to confirm this hypothesis.

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Development and differentiation (or Aging)

OC6.1

Analyses of structural and functional impact of three FGFR3 mutations localized at position K650 leading to both mild and lethal dwarfism

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The fibroblast growth factor receptor 3 (FGFR3) activation leads to dwarfism with a spectrum of severity, hypochondroplasia (HCH), severe achondroplasia with developmental delay and acanthosis nigricans (SADDAN), and thanatophoric dysplasia (TD). Interestingly, FGFR3 mutations localized at the same position in the tyrosine kinase domain are responsible for HCH (p. Lys650Asn), SADDAN (p. Lys650Met) and TD (p. Lys650Glu).
The mechanisms of FGFR3 activation for these three mutants are unknown. To decipher these mechanisms, we developed *in silico*, *in vitro* and *in vivo* studies. Computational studies were conducted to get an atomic description of the p. Lys650Met, p. Lys650Glu and p. Lys650Asn. FGFR3 activation loop mutation-related changes have been quantified by measuring the distance between the activation loop and the C-terminal domain. We demonstrated a correlation within the major modifications and the severity of the dwarfism (Lys650Asn 12.2 Å; Lys650Glu 16.4 Å; Lys650Met 16.8 Å).
To evaluate these structural changes, we transfected three DNA mutants in chondrocyte, we observed a gradient of phosphorylation levels correlated with the severity of the disease. A higher activation of the ERK1/2, AKT, ADAMTS5 and β-catenin signalling pathways was observed in the more severe dwarfism.
We complete these analyses with *in vivo* transient overexpression of *fgfr3* wild type and mutant in zebrafish. At 96hpf injected zebrafish (1 cell stage) present a gradient of skeletal development anomalies correlated with the severity of the dwarfism. 45% of larvae injected with TD mRNA $fgfr3^{Lys650Glu}$ (TD) are highly malformed with axial and craniofacial anomalies whereas mRNA $fgfr3^{Lys650Asn}$ (HCH) injections induce mild skeletal anomalies.
Altogether, the data confirm that the mutation of lysine 650 alters differently the conformation of the kinase domain thus leading to activate unusual signalling pathways and to modify the ossification process. Various biological mechanisms seem to be responsible for mild and lethal dwarfism.

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OC6.2

Bone with uncleavable type I collagen C-propeptide has abnormal development of multiple bone cell populations and increased bone mineral density with age

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Mutations in the C-propeptide cleavage site of both *COL1A1* and *COL1A2* cause dominant high bone mass (HBM) osteogenesis imperfecta (OI), characterized by bone hypermineralization. To elucidate the role of C-propeptide processing in bone formation, we generated heterozygous HBM mice in which both residues of the COL1A1 cleavage site were mutated to prevent cleavage by BMP1. HBM mice are smaller than WT in both weight and length and have extremely brittle bones.
HBM bone extracts contain pC-collagen and increased monomeric COL1A1 C-propeptide. Bone collagen fibrils have a 'barbed-wire' appearance, consistent with the presence of pC-collagen, while dermal fibrils were smaller and more homogeneous than WT with a loss of large fibrils.
Quantitative backscattered electron imaging (qBEI) revealed in cortical femoral bone of 2 month-old mutants an increased bone matrix mineralization: CaMean: +5% ($P = 0.0026$), CaPeak: +6% ($P = 0.0002$); CaHigh +470% ($P = 0.0018$) versus WT. Femoral aBMD is decreased at 2 months but increases to 93% of WT at 1 year. Cortical and trabecular TMD by μCT are decreased versus WT at 2 months, but normalized at 1 year.
Impaired C-propeptide processing affects skeletal geometry and biomechanics. On μCT, HBM femora have thinner cortices and decreased trabecular bone volume. Mechanical testing revealed decreased femoral stiffness, yield and fracture load, with no improvement over time. HBM femora are extremely brittle; post-yield displacement is ~15% of WT ($P < 0.001$). By 6 months, HBM mice hind limb joints are fused with severe osteoarthritis.
C-propeptide processing also influences cellular differentiation of bone. Osteoblast collagen secretion was reduced ~25% in HBM versus WT ($P = 0.023$). Two-month HBM femurs have fewer osteocytes ($P < 0.001$), but they are increased in area ($P < 0.001$). TRAP staining revealed smaller osteoclasts in HBM. These changes in multiple bone cell populations support the prospective signalling function of the C-propeptide trimer, influencing collagenous, cellular and mineral properties of bone.

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OC6.3

The critical biomechanical role of Lipocalin 2 in the crosstalk between endothelium and osteoblasts in unloading conditions

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Angiogenesis and osteogenesis are tightly linked and dependent on each other. Lipocalin 2 (LCN2) is a mechanoresponding adipokine, strongly upregulated in osteogenic cells subjected to microgravity (0.08–0.008 g), in which it impairs osteogenesis and upregulates the osteoclastogenic cytokine, RANKL. We investigated the role of LCN2 in the crosstalk between angiogenesis and osteogenesis in simulated microgravity conditions as a model of mechanical unloading. Mouse and human endothelial cells (EC), subjected to microgravity in the NASA-developed RWV bioreactor, did not express LCN2 but secreted its upstream regulator, IL1β, in their conditioned medium, with a mechanism dependent on microgravity intensity (0.08 g 12 pg/ml; 0.008 g 36 pg/ml, $P < 0.001$). Secreted IL1β induced LCN2 expression in mouse and human osteogenic cells (12.7-fold, $P < 0.001$) by activation and nuclear translocation of the transcription factor NF-κB. LCN2 impaired osteoblast differentiation (−52% ALP $P = 0.024$, −66% mineralization, $P = 0.010$), along with the EC IL1β-induced NOS2/NO/COX2 pathway that increased osteogenic cell proliferation (1.7-fold, $P = 0.003$) and cyclin d1 expression (5.3-fold, $P = 0.002$). Depletion of IL1β from microgravity EC conditioned medium and deletion of LCN2 in osteogenic cells blocked the effect of microgravity on EC-to-osteogenic cell crosstalk. LCN2 was also increased in the conditioned medium of osteogenic cells directly subjected to microgravity (sixfold, $P < 0.001$), and stimulated EC migration (twofold, $P = 0.027$), tube formation (2.3-fold, $P < 0.008$) and sprouting from mouse aortic rings (fourfold, $P < 0.001$). These effects were mediated by LCN2-induced VEGF and Hif1α in EC, and were reduced by LCN2 deletion in osteogenic cells and by incubation with the VEGF receptor antagonist, avastin. Induction of EC-osteogenic cell crosstalk was mediated by these

pathways also ex-vivo, in calvarias cultured in the presence of microgravity EC conditioned medium, and in vivo, in calvarias and tibias injected with microgravity EC conditioned medium, and in tibias from mice subjected to hindlimb unloading by tail suspension or treatment with botulin toxin, suggesting a pathogenic relevance of LCN2-mediated EC/osteogenic cell pathways in conditions inducing disuse osteoporosis.

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OC6.4

Extranuclear effects of estrogen on cortical bone in males is dependent on estrogen receptor A activation function-1

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Estradiol (E_2) signaling via estrogen receptor alpha (ERα) is important for the male skeleton as demonstrated by ERα inactivation in both mice and man. ERα mediates estrogenic effects by translocating to the nucleus and there affects gene transcription, but some effects can also be mediated via extra-nuclear actions of the receptor by, e.g., triggering cytoplasmic signaling cascades. ERα contains various domains and the role of activation function 1 (ERαAF-1) is known to be tissue-specific. The aim of this study was to determine the importance of extra-nuclear estrogen action for maintaining the skeleton in males and to determine the role of ERαAF-1 for mediating these effects. 5-month-old male wild type (WT) and ERαAF-1 inactivated (ERαAF-1^0) mice were orchidectomized (orx) and treated with equimolar doses of 17β-E_2 or 17β-E_2 dendrimer conjugate (EDC), which is incapable of entering the nucleus and thereby only stimulates extra-nuclear ER actions, or their corresponding vehicles for 3.5 weeks. Tibias were analyzed using pQCT. As expected, E_2 treatment increased cortical thickness (+26%, $P<0.001$) and trabecular BMD (+112%, $P<0.001$) in WT mice compared to vehicle treatment. Treatment with EDC resulted in increased cortical thickness in WT mice (+7%, $P<0.05$), while no effect of EDC was detected in the trabecular bone compartment. E_2 treatment increased cortical thickness (+8%, $P<0.05$), but had no effect on trabecular bone in ERαAF-1^0 mice. Interestingly, the effect of EDC on cortical bone was abolished in mice lacking a functional ERαAF-1 domain. In conclusion, extra-nuclear estrogenic signaling is able to enhance cortical bone mass in males and this effect is dependent on a functional ERαAF-1.

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OC6.5

Absence of cyclophilin A impairs endochondral bone formation by altering intracellular signaling pathways required for osteoblast maturation

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Using a CyPB-null mouse model, we previously demonstrated delayed folding, abnormal post-translational modification and altered crosslinking of type I collagen synthesized by osteoblasts. However, intracellular folding of collagen molecules was further delayed by CsA treatment of CyPB-null cells, suggesting involvement of additional PPIases in collagen folding. Since studies of CyPA functions in osteoblasts have not been reported, we investigated the role of this cytoplasmic PPIase in collagen synthesis and bone development using a CyPA knockout mouse model. CyPA$^{-/-}$ mice exhibit moderate growth deficiency, with reduced weight (10%) and decreased (7–9%) femoral and tibial lengths versus WT at age 2 months. Impairment of endochondral bone formation was associated with decreased aBMD in male and female femora (12–14%) and L1/L2 vertebrae (6–12%), respectively. CyPA$^{-/-}$ fibroblasts and osteoblasts secrete normal amounts of type I collagen, with normal electrophoretic mobility. In direct intracellular folding assays, the CyPA$^{-/-}$ fibroblast collagen folding rate was equivalent to WT, and no further delay in folding was observed in CyPA/CyPB double knockout cells compared to CyPB$^{-/-}$ cells alone. Interestingly, Ca^{2+} homeostasis was dysregulated in CyPA$^{-/-}$ cells, resulting in decreased resting cytoplasmic $[Ca^{2+}]_i$, and ATP- and ionomycin-induced Ca^{2+} release from intracellular stores. These findings extend data from platelets demonstrating that the interaction between CyPA and SERCA, the ER calcium replenishment pump, regulates SERCA function. Consequently, expression of both early and late osteoblast markers, including *Rankl*/*Opg*, *Runx2*, *Sp7*, *Alpl*, *Ocn* and *Ibsp,* was decreased 30–80% in differentiating CyPA$^{-/-}$ osteoblasts, while osteoblast expression of adipogenic markers *Cebpβ*, *Cebpδ*, *Pparg*, and target genes *Fabp4* and *Lpl* was increased 200–400% versus WT osteoblasts. Paradoxically, CyPA$^{-/-}$ osteoblasts may activate Wnt signaling, with increased expression of *Wnt5a* (2-fold) and *Axin2* (1.6-fold). Thus, although CyPA has no direct role related to type I collagen, its impact on Ca^{2+}-dependent intracellular signaling pathways affects bone formation by altering osteoblast development.

DOI: 10.1530/boneabs.5.OC6.5

OC6.6

Loss of the longevity gene SirT1 dysregulates chondrocytes and leads to an arthritic phenotype in vivo, via impaired autophagy

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Ageing is universally linked to skeletal deterioration. Common mechanisms may control both processes, where dysregulation may predispose to bone loss and osteoarthritis (OA). The epigenetic modifier SirT1 controls lifespan and decreases with age. However, the role of SirT1 in joint disease is unclear. Human tissue samples, novel genetically modified mice, a surgical disease model and advanced cellular and molecular studies were employed to explore the hypothesis that SirT1 is dysregulated in ageing and OA human cartilage, disrupting normal lifespan-protecting mechanisms such as autophagy, to predispose to OA. Autophagy degrades unwanted proteins, and is defective in ageing. Inhibition of SirT1 (pharmacological and molecular) in HTB94 chondrocytes decreased markers of autophagy (BECN1, ULK-1, LC3, $P<0.01$) and chondrogenesis (COL2A1, ACAN, SOX-9, $P<0.01$). LC3 protein conversion, essential for autophagosome formation, was positively regulated in line with manipulated SirT1 activity in chondrocytes (western blot, FACS ($P<0.001$)) and cartilage explants from LC3-GFP reporter mice ($P<0.01$). This demonstrates that SirT1 alters autophagic flux, whilst IP analysis showed direct mechanistic binding of SirT1 with autophagy proteins.

Human OA cartilage showed decreased SirT1 compared to healthy cartilage ($P<0.05$). Similarly, murine models with a novel articular cartilage-specific SirT1 deletion (SirT1$^{fl/fl}$x Aggrecan CreER^{T2} mice (SirT1^{Agg})), showed significant cartilage degradation, increased OA disease score and epiphyseal volume (vs WT, $P<0.001$) on histomorphometric and uCT analysis at 2, 6, 12 months. SirT1Agg mice also showed decreased ACAN, COL2A1, SOX-9 ($P<0.001$) and autophagy markers BECN1, ULK-1, LC3, ATG5, ATG7, ATG13 ($P<0.01$), decreased LC3 protein in hip explants ($P<0.01$), and decreased autophagosome number in microdissected cartilage (SEM, $P<0.05$). Induction of experimental OA (destabilisation of the medial meniscus surgery) exacerbated cartilage degradation in SirT1Agg mice vs WT, increased OA disease score and decreased LC3 staining.

These studies suggest that declining SirT1 in ageing human cartilage, or SirT1 deletion in murine chondrocytes, results in OA due to dysregulated autophagy in chondrocytes.

DOI: 10.1530/boneabs.5.OC6.6

Oral Posters

Clinical

OP1

Effects of calcifediol versus cholecalciferol on 25(OH)D3 serum levels, appendicular muscle strength, and physical performance in post-menopausal women

Antimo Moretti, Alessandro de Sire, Dario Calafiore, Raffaele Gimigliano, Francesca Gimigliano & Giovanni Iolascon

see P260.

DOI: 10.1530/boneabs.5.P260

OP2

Abstract withdrawn.

DOI: 10.1530/boneabs.5.P272

OP3

Vitamin D supplementation for 12 months in older people prevents bone loss and suppresses parathyroid hormone levels

Terry J Aspray, Roger M Francis, Elaine McColl, Thomas Chadwick, Elaine Stamp, Ann Prentice & Inez Schoenmakers

see P221b.

DOI: 10.1530/boneabs.5.P221b

OP4

Identification of a novel locus on 2q13 of large effect size which predisposes to clinical vertebral fractures independently of BMD: the GEFOS consortium

Nerea Alonso, The Clinical Vertebral Fracture Consortium, Andre Uitterlinden, Fernando Rivadeneira & Stuart H Ralston

see P241.

DOI: 10.1530/boneabs.5.P241

OP5

Predicting hip fracture type of elderly Asian patients with low-energy fall by volumetric BMD and femoral morphology from QCT

Yong-Bin Su, Xiao-Guang Cheng, Ling Wang & Yi-Min Ma

see P296.

DOI: 10.1530/boneabs.5.P296

OP6

Gender-different relationship between body composition and incident fracture risk in Koreans: a community-dwelling prospective cohort study

Jung Hee Kim, A Ram Hong, Hyung Jin Choi, Eu Jeong Ku, Nam H Cho & Chan Soo Shin

see P368.

DOI: 10.1530/boneabs.5.P368

OP7

Can bone turnover markers help to define the duration of bisphosphonate drug holidays?

Louise Statham, Terry Aspray & Sharon Abdy

see P410.

DOI: 10.1530/boneabs.5.P410

OP8

Two-fold regional variation in initiation of anti-osteoporosis medication after hip fracture in the UK

Anjali Shah, Daniel Prieto-Alhambra, Samuel Hawley, Antonella Delmestri, Janet Lippett, Cyrus Cooper, Andrew Judge & Kassim Javaid

see P401.

DOI: 10.1530/boneabs.5.P401

OP9

Short-term smoking cessation improved bone formation in healthy male smokers

Reiko Watanabe, Nobuyuki Tai, Junko Hirano, Hiroaki Masaki, Ryo Okazaki & Daisuke Inoue

see P342.

DOI: 10.1530/boneabs.5.P342

Pre-Clinical

OP10

Pathophysiological implication of Autotaxin on osteoclast function

Sacha Flammier, Tristan Gicquel, François Duboeuf, Olivier Peyruchaud, Fabienne Coury & Irma Machuca-Gayet

see P189.

DOI: 10.1530/boneabs.5.P189

OP11

Phosphorylation of S122 in ERα is important for the skeletal response to estrogen treatment

Karin Gustafsson, Helen Farman, Vikte Lionikaite, Jianyao Wu, Petra Henning, Annica Andersson, Ulrika Islander, Angelina Bernardi, Sara Windahl, Sofia Skrtic, Klara Sjögren, Antti Koskela, Juha Tuukkanen, Andree Krust, Pierre Chambon, Claes Ohlsson & Marie Lagerquist

see P490.

DOI: 10.1530/boneabs.5.P490

OP12

Loss of androgen receptor suppresses chondrogenic proliferation during endochondral ossification in mice

Hong-Yo Kang, Yun-Ju Chen, Ko-En Huang & Chawnshang Chang

see P58.

DOI: 10.1530/boneabs.5.P58

OP13

The CAM assay for human bone regeneration evaluation: the potential of Laponite® clay gel for growth factor delivery *ex vivo*

Ines Moreno-Jimenez, Gry Hulsart-Billstrom, Stuart A Lanham, Janos M Kanczler, Nicholas D Evans & Richard O C Oreffo

see P487.

DOI: 10.1530/boneabs.5.P487

OP14

Loss of type I collagen telopeptide lysyl hydroxylation causes musculoskeletal abnormalities in a zebrafish model of Bruck syndrome

Andy Willaert, Charlotte Ghistelinck, P Eckhard Witten, Ann Huysseune, Pascal Simoens, Sofie Symoens, Fransiska Malfait, Amelie De Muynck, Anne De Paepe, Ronald Y Kwon, Mary Ann Weiss, David E Eyre & Paul Coucke

see P483.

DOI: 10.1530/boneabs.5.P483

OP15

Two different domains of fibronectin stimulate osteoblast differentiation by activating distinct integrins

Carla Sens, Katrin Rau & Inaam Nakchbandi

see P168.

DOI: 10.1530/boneabs.5.P168

OP16

Bcl-2-associated athanogene-1 (BAG-1) regulates chondrocyte and osteoblast development

Joanna Greenhough, Emmanouil Papadakis, Ramsey Cutress, Paul Townsend, Richard Oreffo & Rahul Tare

see P62.

DOI: 10.1530/boneabs.5.P62

OP17

Transgene expression by Dmp1 promoter fragments occurs in various organs

Hiroaki Saito, Hanna Taipaleenmäki, Ahmed Al-Jazzar, Andreas Gasser, Behzad Javaheri, Cheryl Scudamore, Teresita Bellido, Andrew A Pitsillides & Eric Hesse

see P208.

DOI: 10.1530/boneabs.5.P208

OP18

Osteocalcin transgenic mice reveal aspects of the bone/glucose axis, as well as powerful suppression of ectopic osteocalcin protein production

Harry Horsnell, Natalie Wee, Rishikesh Kulkarni, Herbert Herzog & Paul Baldock

see P231.

DOI: 10.1530/boneabs.5.P231

Clinical

OP19

Profiles of 25 hydroxyvitamin D and its metabolites 24, 25-dihydroxyvitamin D and 1, 25-dihydroxyvitamin D in vitamin D_3 supplementation studies

Jonathan Tang, Holly Nicholls, John Dutton, Isabelle Piec, Christopher Washbourne, L Saleh, A Novak, G Close, H Macdonald, S Jackson, J Greeves & William Fraser

see P21.

DOI: 10.1530/boneabs.5.P21

OP20

Longitudinal increase in vitamin D binding protein levels after initiation of tenofovir/lamivudine/efavirenz therapy among HIV-infected individuals

Evelyn Hsieh, Liana Fraenkel, Yang Han, Weibo Xia, Karl Insogna, Michael Yin, Ting Zhu, Xinqi Cheng & Taisheng Li

see P337.

DOI: 10.1530/boneabs.5.P337

OP21

Impact of 3-year vitamin D and calcium supplementation on mineral and organic matrix formation of trabecular bone in postmenopausal osteoporosis

E P Paschalis, S Gamsjaeger, N Hassler, A Fahrleitner-Pammer, H Dobnig, J J Stepan, E F Eriksen & K Klaushofer

see P407.

DOI: 10.1530/boneabs.5.P407

OP22

Trabecular (spine) bone density increases significantly in the first six months after weaning (factors affecting bone formation after breastfeeding pilot study (FABB-Pilot))

Sandra Cooke-Hubley, Gerry Mugford, James Valcour, Michael Wahl, Janine Woodrow, J. D. Adachi & Christopher S. Kovacs

see P75.

DOI: 10.1530/boneabs.5.P75

OP23

FGF23 and SCL are expressed in carotid plaques and the association between their circulating fractions and fractures differs in relation to comorbidity in elderly individuals

Stefano Rotatori, Claudio Corallo, Daniela Merlotti, Domenico Rendina, Simone Bianciardi, Aurora Patti, Stefano Gonnelli, Isabella Anna Evangelista, Barbara Lucani, Maria Beatrice Franci, Carlo Setacci, Pasquale Strazzullo, Ranuccio Nuti, Francesco Dotta & Luigi Gennari

see P367.

DOI: 10.1530/boneabs.P367

OP24

Secondary bone size deficit in patients with Ehlers–Danlos syndrome

Charlotte Verroken, Patrick Calders, Inge De Wandele, Fransiska Malfait, Hans Zmierczak, Stefan Goemaere, Jean-Marc Kaufman, Bruno Lapauw & Lies Rombaut

see P462.

DOI: 10.1530/boneabs.P462

OP25

Validation of a novel scoring system, the radiographic global impression of change (RGI-C) scale, for assessing skeletal manifestations of hypophosphatasia in infants and children

Michael Whyte, Kenji Fujita, Scott Moseley, David Thompson & William McAlister

see P475.

DOI: 10.1530/boneabs.5.P475

OP26

Atypical femur fractures (AFF): a case-control study

Erik Imel, George Eckert, Katie Allen, Julie Chandler, Joel Martin, Siu Hui, C Conrad Johnston, Anne DePapp, Art Santora, Robert Choplin, Trenton Roth & Ziyue Liu

see P352.

DOI: 10.1530/boneabs.5.P352

OP27

Acute kidney injury after a single intravenous zoledronic acid administration in patients with osteoporosis

Cristiana Cipriani, Carolina Clementelli, Valeria Fassino, Rizieri Manai, Vittoria Danese, Veronica Cecchetti, Federica Ferrone, Jessica Pepe & Salvatore Minisola

see P395.

DOI: 10.1530/boneabs.5.P395

Pre-Clinical

OP28

Neuropetide Y Y_1 receptor deletion impairs matrix demineralization and resorption

Daniela M Sousa, Francisco Conceição, Luis Leitão, Estrela Neto, Cecília J Alves, Inês S Alencastre, Herbert Herzog, Paulo Aguiar & Meriem Lamghari

see P194.

DOI: 10.1530/boneabs.5.P194

OP29

Hif1alpha leads to chondrodysplasia in MMP-deficient mice
Claire-Sophie Devignes, Oriane Duchamp de Lageneste, Alexis Gonon, Audrey Devillers, Ying Yu, Zena Werb & Sylvain Provot

see P214.

DOI: 10.1530/boneabs.5.P214

OP30

Bone loss in KLHL3 knock-in mice characterized by a pseudohypoaldosteronism type II-like phenotype is mediated by renal PTH resistance
Olena Andrukhova, Jinwei Zhang, Dario Alessi & Reinhold Erben

see P90.

DOI: 10.1530/boneabs.5.P90

OP31

Early deletion of menin in the osteoblast lineage results in decreased bone mass in adult mice
Jad Abi Rafeh, Lucie Canaff & Geoffrey Hendy

see P151.

DOI: 10.1530/boneabs.5.P151

OP32

Pharmacological activation of the non-canonical TGF-β signaling is a novel strategy to enhance bone formation
Abbas Jafari, Majken Siersbaek, Li Chen & Moustapha Kassem

see P146.

DOI: 10.1530/boneabs.5.P146

OP33

Long-term high-dose resveratrol supplementation reduces bone mass and strength in rats
Marie Juul Ornstrup, Annemarie Brüel, Jesper Skovhus Thomsen, Torben Harsløf, Bente Lomholt Langdahl & Steen Bønløkke Pedersen

see P393.

DOI: 10.1530/boneabs.5.P393

OP34

RCOR2 is a novel regulator of osteoblast differentiation
Kati Tarkkonen, Rana Al Majidi, Cristina Valensisi, Lauri Saastamoinen, David Hawkins & Riku Kiviranta

see P162.

DOI: 10.1530/boneabs.5.P162

OP35

Identification and functional validation of microRNA expression in human bone tissue
Barbara Ostanek, Simona Mencej Bedrac, Barbara Kern, Peter Vrtacnik, Vid Mlakar, Tamer Bego, Radko Komadina, Rihard Trebše & Janja Marc

see P248.

DOI: 10.1530/boneabs.5.P248

OP36

MicroRNA-125b in bone matrix plays a crucial role in osteoblast-osteoclast communication
Tomoko Minamizaki, Yuichiro Takei, Yuko Nakao, Yasumasa Irie, Hirotaka Yoshioka, Kotaro Tanimoto, Katsuyuki Kozai & Yuji Yoshiko

see P85.

DOI: 10.1530/boneabs.5.P85

Poster Presentations

Arthritis and Other Joint Diseases: Translational and Clinical

P1

The role of fibroblast growth factor in the destruction in rheumatoid arthritis

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The main cause of the bone destruction in rheumatoid arthritis (RA) is a destructive act of aggressively growing pannus, growth and angiogenesis of which are caused by the proliferation of fibroblasts in the synovium (S) due to the activation of fibroblast growth factor (FGF).

Objectives

To reveal relationships of FGF with ultrasonic and arthroscopic and proliferative-destructive histological figures in RA.

Methods

Complete comprehensive clinical laboratory and instrumental examination was performed in 128 patients with RA (ACR/EULAR 2010), the concentration of FGF in the blood was determined by enzyme immunoassay. Ultrasound of the knee joint was performed on the device 'ESAOTE MyLAB40', knee arthroscopy was performed using the arthroscope (Karl Storz GmbH), the biopsy of S was taken from the three most changed areas, samples were fixed in 10% buffered formalin, stained with hematoxylin and eosin, used the microscope Axiostar (Carl Zeiss).

Results

There were direct correlations the FGF with ultrasonic characteristic of the S thickness, with the presence of pannus and cartilage-bone erosions ($P<0.001$ in all cases). There was also a direct relation with the S vascularization intensity index ($R=0.31$, $P=0.03$). Indicator FGF had direct correlations with indicators of S macro-asssessment: arthroscopic – villous hyperplasia and the presence of pannus ($P<0.001$); and also with the parameters of S micro-assessment: histological – hyperplasia of the villi with the covering cell proliferation ($P<0.01$), mucoid swelling and fibrinoid changes ($P<0.05$).

Conclusion

The high concentration of FGF in the blood is related to the worsening of proliferative and destructive indices in RA, namely the degree of vascularization and thickening of S, pannus and cartilage-bone erosions formation. There for high FGF level in the blood of RA patients may be regarded as a marker of the early development of bone destruction.

DOI: 10.1530/boneabs.5.P1

P2

Does adiponectin in serum or synovial fluid predict arthroscopy assessed cartilage damage severity in patients with symptomatic knee osteoarthritis?

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Background

Osteoarthritis (OA), the prevalent joint-affecting disease characterized by cartilage damage, is the leading cause of disability in adults and contributes to the excess of morbidity and healthcare costs. We performed the current trial to evaluate biomarkers, specifically adiponectin in serum and synovial fluid, associated with cartilage damage severity assessed by arthroscopy, in patients with symptomatic knee osteoarthritis.

Methods

The 40 subjects (mean age 51.8, 35% female, mean BMI 28.8) were divided into two groups according to arthroscopy assessed cartilage damage, using Outerbridge (OB) grading: Group 1 included 20 patients without cartilages surface defects (OB grade 0, I), Group 2 included 20 patients with cartilages surface defects (OB grade II, III). Metabolic parameters, insulin resistance markers and serum adiponectin levels were determined.

Results

Both groups were similar in terms of serum adiponectin levels ($P=0.806$). Synovial fluid adiponectin levels tended to be lower (not statistically significant) in subjects with cartilage damage (1718.6 vs. 2738.1). Knee Society Score was significantly lower in subjects with cartilage damage (113.0 ± 24.9 vs. 142.7 ± 25.1, $P<0.001$). In multiple linear regression analysis BMI was a significant independent determinant of cartilage damage in non-obese patients with knee osteoarthritis, such that each one-unit increase in BMI was associated with a 21.7% increase in risk of cartilage damage (OR1.217, 95% CI 0.998–1.483, $P=0.05$).

Conclusions

We did not find an association between serum adiponectin as well as adiponectin in synovial fluid, and arthroscopy assessed cartilage damage severity. BMI was a significant independent determinant of cartilage damage in non-obese patients with knee osteoarthritis.

DOI: 10.1530/boneabs.5.P2

P3

The influence of secondary hyperparathyroidism -at the time of index operation- on the later development of (septic or aseptic) loosening of implants in female patients with knee osteoarthritis who undergo total knee arthroplasty

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Objectives

Prospective case-controlled study assessing whether the incidence of loosening of implants in patients undergoing Total Knee Arthroplasty (TKA) is higher among those with Secondary Hyperparathyroidism (SP) at the time of index operation.

Material and methods

Caucasian female patients with knee osteoarthritis who underwent TKA between November 2004 and March 2007 were enrolled. Exclusion criteria: endocrinopathies, rheumatoid or secondary arthritis, renal disease, fracture or orthopaedic surgery 24 months before enrollment. Patients with osteopenia/osteoporosis were not excluded. Serum intact PTH (I-PTH), calcium, phosphorus, creatinine, and the clearance of creatinine were determined pre-operatively. Case-notes were reviewed for any revision operation.

Results

Two hundred and eighty-three women aged 49–81 (mean 70) were enrolled; 100 had abnormally elevated I-PTH. Two with primary hyperparathyroidism were excluded from analysis. The incidence of SH was 35%. I-PTH correlated positively with age ($P=0.008$) and creatinine level ($P=0.021$) and negatively with the clearance of creatinine ($P=0.004$). In multiple regression analysis, 7.3% of the variance in I-PTH values ($R^2=0.073$, $P<0.001$) was significant; creatinine level was the largest contributor (standardised $\beta=0.275$, $P=0.08$). 265 patients were available for re-evaluation at an average follow-up period of 115 (105–132) months. Three patients with SH and seven with normal I-PTH values (at the index operation) were re-operated due to aseptic loosening at an average period of 51.3 (26–90) and 49.9 (25–94) months respectively. The difference between the number of re-operated patients with pre-operative SH and normal I-PTH values, was statistically non-significant ($P>0.005$). The mean time to re-operation was also non-significant ($P>0.005$). One patient with SH and two with normal I-PTH, were re-operated due to septic loosening ($P>0.005$). The mean time to revision operation was 7.6 (4–12) months.

Conclusion(s)

Our results show that SH does not enhance aseptic loosening of implants in TKA. Larger series are needed, especially as far as septic loosening and SH is concerned.

DOI: 10.1530/boneabs.5.P3

P4

Immune system and bone cells in early rheumatoid arthritis

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Rheumatoid arthritis (RA) is an immune-mediated disease characterized by T cells activation and bone erosions that severely reduces patients quality of life; however a clear role for different T helper (Th) cells has not been established yet. This work aims to evaluate Th phenotypes, osteoclast (OCs) precursors cells and cytokines in peripheral blood of women affected by early RA (eRA) compared to healthy women matched for age. The study was approved by our Ethical Committee.

We enrolled in the study 36 women affected by eRA and 31 healthy controls. To quantify OCs precursors and Th subset in peripheral blood we used flow cytometry. We measured classical OCs precursors (CD14+/CD11b+/VNR+) and inflammatory OCs precursors (CD14+/CD11b+/VNR+/CD16+) and Th subset. We also measured TNFα, IFNγ, TGFβ, IL-4, IL-17, IL-23, IL-6, RANKL and OPG in the serum by ELISA.

Th were significantly altered in eRA: Th17 and Th17/IFNγ were increased, whereas Th1, Th2 and Tregs were not significantly affected. Classical OCs were reduced in eR patients whereas inflammatory OCs were increased and correlated with CRP (Rho 0.35, $P=0.039$).
RANKL/OPG was significantly increased in eRA due to increased RANKL and directly correlated with inflammatory OCs precursors. ($R=0.31$, $P=0.017$).
TNFα, TGF-β, IL-23 and IL-6 were significantly increased whereas IL-17 and IFNγ were not.
The increase in inflammatory OC precursors suggests a specific role for these cells in bone erosions and possibly in systemical bone loss in RA.
This is the first attempt to describe Th cells and OC precursors in early phase of RA.
We showed an impairment in Th cells subtypes that may be the first pathogenetic driver in early phase of RA whereas the increase in TGFβ may be due to deregulation in T regulatory cells function. Previous paper showed conflicting results on Th subsets in RA, this is mainly due to different models used.

DOI: 10.1530/boneabs.5.P4

P5

The effects of hydroxychloroquine on bone turnover

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Introduction
We recently showed that patients with primary Sjögren Syndrome (pSS) have significantly higher bone mineral density (BMD) in the lumbar spine and femoral neck compared with healthy controls. The majority of those patients (69%) were using hydroxychloroquine (HCQ), which may have favourable effects on BMD.
Aim
To evaluate whether HCQ modulates human bone cells *in vitro*.
Methods
Osteoblasts were differentiated from human mesenchymal stromal cells. We measured alkaline phosphatase (ALP) for osteoblastic differentiation and calcium incorporation as a measure for mineralization at day 7 and 18, respectively. Osteoclasts were cultured from peripheral blood mononuclear cells. At day 14 of culture, the osteoclasts were counted using tartrate-resistant acid phosphatase staining and osteoclast activity was measured using von Kossa staining of hydroxyapatite-coated surfaces. All cultures were treated with different HCQ doses (control, 0.2, 1 and 5 μg/ml). Additionally, polymerase chain reaction (PCR) technique was used for osteoblast gene expression.
Results
We observed that osteoblast differentiation decreased dose-dependently by HCQ with a 3.1-fold decrease between the highest dose and controls as assessed by ALP measurements. Also, mineralization of these cultures diminished by increased HCQ doses. Using the highest HCQ dose almost no mineralization was observed. PCR analysis showed a dose-dependent decrease of osteopontin and osteocalcin expression.
Osteoclast numbers were also decreased (1.9-fold) following 5 μg/ml HCQ treatment. This was reflected by strongly reduced bone resorption as assessed by resorption pit number (3.5-fold) and resorption surface (9.0-fold).
Conclusion
We demonstrate that HCQ is suppressing both bone formation and resorption. Based on the clinical data we assume that HCQ is favouring bone formation, but additional work is required to evaluate the contribution of bone resorption and formation to the observed phenotype. Therefore, the use of HCQ as an explanation for the higher BMD in pSS patients may be the consequence of reduced bone turnover.

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P6

Effects of 17β-estradiol and mechanical overloading on osteoarthritis of rat temporomandibular joint

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Objectives
Sex hormones and mechanical overload have been regarded as main causative factors in the pathogenesis of temporomandibular joint (TMJ) osteoarthritis. However, there was no report regarding the relevance of mechanical overload on the mandibular condyle with serum estrogen level. This study aimed to investigate changes of the mandibular condyle of TMJ in ovariectomized rat under increased loading, and to evaluate the inhibitory effect of systemic administration of 17β-estradiol (E2) on the degenerative change of mandibular condyle.
Methods
Female Sprague-Dawley rats were mechanically loaded 8 weeks after ovariectomy (OVX) by connecting the mandibular angle and zygomatic arch using orthodontic elastic with a force of 50 g. Exogenous E2 (500 μg/kg) was subjected to daily intramuscular injection for 28 days on induced TMJ arthritis after mechanical load and OVX. Changes in the condyles were analyzed using micro-CT, histochemical/immunohistochemical staining 12 weeks after OVX.
Results
OVX induced the reduction of size, and osteoporotic change in the condyles. OVX led to more obvious changes of the microarchitecture parameters at the posterior area of the condyle with reduced bone mineral density and bone volume/tissue volume (BV/TV). Incidence of arthritis of the mandibular condyles was 25.0 and 61.9% without and with loading in OVX rats, respectively. The mandibular condyle of loaded-OVX rats showed a tendency toward reduced AP length. E2 administration recovered osteoporotic change in the mandibular condyles but did not arthritic changes in the short term. Immunohistochemically, loaded group increased the expression of tumor necrosis factor-α or estrogen receptor-α in the presence of E2.
Conclusions
Current findings showed that the synergistic effects of estrogen deficiency and mechanical overloading on the mandibular condyles are probably main contributing factors for the occurrence of TMJ arthritis. Systemic estrogen therapy might reverse the osteoporotic changes of the mandibular condyle, suggesting an intriguing alternative to improve the degenerative change.

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P7

Effect of estrogen deficiency on loaded and non-loaded area of temporomandibular or knee Joint

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Introduction
Low level of estrogen has regarded as a main contributing factor of temporomandibular joint (TMJ) arthritis in young women patients. However, there is lack of evidence about the occurrence of arthritis in knee joint (KJ) related with estrogen deficiency. This study aimed to investigate the effect of estrogen deficiency on the loaded and non-loaded bone area of TMJ or KJ.
Method and materials
Total of 28 SD rats were allocated into two groups, the sham surgery group and the ovariectomy (OVX) group. TMJ was subjected to mechanical loading with elastic power chain between mandibular angle and zygoma in half of animals. At 12 weeks after OVX, all groups were scarified. Changes in the bone area of KJ and TMJ were analyzed using micro-computertomography (micro-CT).
Results
We analyzed the bone area of joint region in TMJ and KJ each which was compartmentalized into three areas on loaded, middle and non-loaded area. Bone mineral density (BMD) and three-dimensional micro-CT parameters were compared between TMJ and KJ. Non-loaded area of TMJ showed a significant decrease in bone volume/tissue volume (BV/TV) and BMD at ovariectomized rats, which was independent of mechanical loading. However, there was no difference in BV and BMD either in loaded area of TMJ or in all areas of KJ in both loaded and non-loaded OVX rats. Middle area of TMJ in OVX rats with or without loading showed a significant decrease in BV/TV, but no difference in BMD.
Conclusion
These results revealed that OVX-mediated estrogen deficiency led to a significant decrease of bone formation and quality in non-loaded bone area of TMJ and no influence on bone area of KJ, suggesting that TMJ is more sensitive to estrogen deficiency.

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P8

A clinical study to examine thresholds of joint space width and joint space area for identification of knee osteoarthritis

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Objective
Osteoarthritis (OA) is a degenerative, slowly developing joint disease. Although clinical indications of OA can vary among different definitions there is a general agreement that the disease is associated with cartilage narrowing. However, there is no general consensus about the threshold below which the joint space width (JSW)/joint space area (JSA) can be certain indicators for the state of OA. Therefore this study evaluates these limits to reveal quantitative information about indicators of OA.
Methods
The study included 226 standardized 2D knee radiographs from 101 cases and 125 controls. All images were acquired in PA direction and standardized positions. The minimum JSW and JSA were calculated by using the i3a software. Three physicians assessed by using the Kellgren & Lawrence Score and assigned the images to either a Case or Control group. A knee was assigned to the Case group, if at least two physicians assessed it as being affected by OA.
Results
Considering the minimum JSW, an odds ratio of 5.63 (CI: 3.17–9.99) with an accuracy of 70.35% and a sensitivity of 70.30% can be obtained. Every subject that has a minimum JSW below 3.4 mm belongs to Cases. With respect to the minimum JSA, the odds ratio is 3.60 with an accuracy of 65.49% and a sensitivity of 65.35%. Results also show that every subject with a minimum JSA below 50 mm^2 is being considered to have OA.
Conclusion
Based on this study it can be concluded that a JSW below 3.4 mm and a JSA below 50 mm^2 at the knee joint are strong indicators for OA. Thus, for clinical assessments it is suggested to consider these threshold values for diagnostic purposes. In further studies, symptomatic knee OA should be incorporated to verify whether minimum JSWs and JSAs can also be linked to symptomatic knee pain.

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P9

Apolipoprotein E aggravates inflammation and bone destruction in murine antigen – induced arthritis

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Background and Objectives
Rheumatoid arthritis (RA) is a chronic disease characterized by severe bone destruction which has been associated with altered lipid metabolism. Apolipoprotein E (Apo E) is a lipoprotein mainly produced by macrophages. ApoE has been described as crucial in lipid metabolism but also as an important anti-inflammatory mediator regulating innate immunity and bone turnover. In the present study we investigated the role of Apo E in bone destruction during antigen-induced arthritis (AIA).
Methods
Experimental arthritis (AIA) was induced by injection of 60 µg mBSA into the knee joint of $ApoE^{-/-}$ and wild type (WT) control mice previously immunized with mBSA/CFA.
Joint swelling was measured by uptake of ^{99m}Technecium (^{99m}Tc) and expressed as a ratio of the uptake in right (injected) knee joint and the left (non injected). Humoral immunity (mBSA antibody titer) was measured by ELISA. Joint inflammation and bone erosion were measured by histological analysis using an arbitrary scale from 0 to 3. $TRAP^+$ cells were determined using immunohistochemistry.
Results
$ApoE^{-/-}$ mice showed significantly less joint swelling at day 1, 3 and 7 after AIA induction compared to WT controls (21, 17, 18% lower, respectively). Serum level of specific anti mBSA (total IgG, IgG1, IgG2a and IgG2b) was comparable between the two mouse strains. At day 21 histology of the knee joints showed less infiltration of inflammatory cells (25% lower) and decreased bone erosion in the $ApoE^{-/-}$ mice compared to WT controls (25% lower). In line with that, $ApoE^{-/-}$ mice revealed a reduction of the number of osteoclasts present at the resorbed area (36% lower), measured by image analysis of TRAP staining.
Conclusions
ApoE aggravates bone destruction in AIA by increasing influx of inflammatory cells within the synovium and the number of resorbing osteoclasts along the bone.

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P10

Identifying cell populations coupling inflammation to osteoresorption in arthritis

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Rheumatoid arthritis (RA) is characterised by periarticular bone destruction leading to permanent disability. Some other forms of arthritis, such as arthritis in systemic lupus erythematosus (SLE), rarely produce bone lesions. Corresponding to that, inactivation of Fas produces a murine SLE-like phenotype, but alleviates bone loss during antigen-induced arthritis (AIA). Aim of this study was to identify cell populations differentially regulated in non-resorptive vs. resorptive arthritis by comparing the synovial and bone marrow cellular composition in Fas-deficient and wild-type mice with AIA.
After obtaining approval from the Ethical Committee, mice were immunized with methylated(m)BSA in complete Freund's adjuvant, followed by intra-articular injection of mBSA. Five weeks post-immunization, arthritis was assessed by histology and µCT. After collagenase digestion and labelling, cellular phenotype was determined by flow cytometry for the following markers: CD3, CD4, CD8, CD11b, CD29, CD31, CD44, CD45, CD90.1, CD106, CD115, CD166, CD117, B220, Gr-1, Sca-1, and TER119.
Micro-CT confirmed pronounced decrease in epiphyseal subchondral trabecular bone volume in wt mice with AIA ($22.31 \pm 3.74\%$), in comparison to control group ($31.18 \pm 3.14\%$, $P=0.002$, t-test), and the decrease was absent in Fas $-/-$ mice ($29.57 \pm 3.07\%$ in control vs. $27.98 \pm 4.08\%$ in AIA, $P=0.60$, t-test). Proportions of B220+, CD3+ and CD11b+ cells were significantly increased ($P=0.04$, $P=0.008$, $P=0.019$, respectively, Kruskal-Wallis test), while the proportions of CD106+ and CD166+ non-haemopoietic cells were significantly decreased in the synovial compartment of wild-type mice with AIA ($P=0.04$, and $P=0.05$, respectively, Kruskal-Wallis test). In all mice with AIA, proportions of CD3+, CD11b+ and Gr-1+ cells were strongly negatively associated with bone volume ($\rho < -0.60$, $P<0.05$), whereas positive association was found for CD106+ and 166+ stromal cells ($\rho > 0.60$, $P<0.05$).
Populations specifically altered in non-resorptive form of arthritis are potentially involved in coupling the inflammatory process to bone destruction. Further analysis of their molecular signatures may identify novel targets for counteracting osteoresorption induced by inflammation.

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P11

Chemotactic signals mediating osteoclast progenitor migration in collagen induced arthritis

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Introduction
Collagen induced arthritis (CIA) is a mouse model of rheumatoid arthritis marked by persistent inflammation and enhanced osteoresorption at the affected joints as well as systemic osteopenia due to increased activation of osteoclast progenitors (OCP). Abundantly secreted chemokines presumably attract OCPs to the inflamed sites. Our goal was to identify chemotactic signals important for OCPs recruitment in CIA.

Methods
After obtaining approval from the Ethical Committee, C57BL/6 mice were immunized with chicken type II collagen in Freund's adjuvant. Arthritis development was confirmed by clinical scoring and anti-collagen antibody detection. To determine OCP phenotype, peripheral blood mononuclear cells (PBMCs), bone marrow and collagenase digested tarsometatarseal joints were assessed by series of markers (CD3, B220, NK1.1, CD45, CD11b, CD115, CCR2, CCR5, CCR9, CXCR4) using flow-cytometry. For *in vitro* migration assay, PBMCs were stimulated with M-CSF and RANKL, seeded into transwell inserts and analyzed for the migration potential toward CCL2 or CCL5 contained in the lower chambers.
Results
Immunized mice developed arthritis with the incidence of about 60%. Frequency of OCPs contained among CD3-B220-NK1.1-CD45+CD11b+CD115+ population was increased in CIA (3.4±0.13% in control vs 7.02±2.59% in CIA, $p < 0.05$). Chemokine receptors were expressed by certain proportion of OCPs in both groups, with substantial expression of CCR2 (27.95±5.93%) and CCR5 (24.45±5.35%), and variable expression of CCR9 (9.69±8.30%) and CXCR4 (1.13±1.04%). OCPs from mice with CIA showed increased migration potential toward chemoattractant gradients (median 11.5 (IQR 11 to 13.25) migrated cells in control vs 20.5 (IQR 20–25) in CIA for CCL2, $P < 0.05$, and 12.5 (IQR 9–18.5) in control vs 33.5 (IQR 23–35.75) in CIA for CCL5, $P < 0.05$).
Conclusions
Our results indicate that CCL2/CCR2 and CCL5/CCR5 signaling possibly contribute to increased OCP migration. Therapeutic blocking of such chemotactic signals represents a promising approach to antagonize enhanced osteoresorption in inflammatory diseases.

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Biochemical testing

P12

Improvement on growth of osteoporotic bone tissue around screw and fixation strength of screw induced by stress force from the expanding pedicle screw: dynamic microstructural, histological and biomechanical studies in osteoporotic sheep lumbar vertebrae

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Objective
It was proved that expansive pedicle screw (EPS) could significantly enhance immediate screw stability through mechanical expanding and compressing. However, there were little researches on growth of bone tissue around screw and dynamic screw stability *in vivo* in osteoporotic vertebrae. This study was designed to evaluate growth and microstructure of bone tissue around screw and fixation strength of screw in osteoporotic sheep lumbar vertebrae.
Method
Osteoporotic sheep model was established through bilateral ovariectomy and methylprednisolone injection. Conventional pedicle screw (CPS) and EPS were randomly inserted through pedicle into vertebral body. Sheep were sacrificed at both 6-week and 12-week post-operation. Biomechanical tests were performed to evaluate screw stability and micro-CT analysis and histological observation were performed to evaluate microstructure and growth of bone tissue around screw.
Results
The bone trabeculae around expanding part of EPS were more and denser than those around CPS,and the microstructural parameters of bone trabeculae around EPS were significantly improved compared with those around CPS at both 6-week and 12-week. From 6-week to 12-week, more and more bone trabeculae surrounded screw, crept into interspaces between two fins and connected with each other forming mature structure of cavitas medullaris around screw. At 12-week mature bone tissue took place of fibrous tissue at 6-week and formed biological interface improving quality of bone tissue around anterior part of EPS. Those parameters of EPS at 12-week were significantly improved compared those at 6-week. At both 6-week and 12-week, stabilities of EPS were significantly higher than that of CPS. The stability of EPS at 12-week was significantly higher than that at 6-week.
Conclusions
EPS showed excellent stability and biological interface compared with CPS *in vivo* in osteoporosis. With continuous expanding and compressing of EPS, microstructure of bone tissue around EPS and stability showed further significant improvement in long-term.

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P13

Evaluation of serum levels progranulin and bone morphogenetic protein-4 in patients with osteoarthritis

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Osteoarthritis (OA) is a chronic, slowly progressive disease of the joints and is one of the most common causes of pain and disability in middle-aged and older people. The etiology and pathogenesis underlying this disease are poorly understood. Progranulin (PGRN), a secreted glycoprotein expressed in many cell types, has been linked to wide variety of biological processes. In recent years, increasing evidence suggests that PRGN stimulates chondrocyte proliferation and is considered an essential regulator of cartilage metabolism. Bone morphogenetic protein-4 (BMP-4), a member of transforming growth factor-β superfamily of proteins, is involved in bone and cartilage development and induces chondrogenesis. This study aimed to investigate serum BMP-4 and PRGN levels in patients with OA and present a new evidence of pathogenesis OA disease. The study included 38 female osteoarthritis patients and 38 female healthy volunteers. Serum PRGN and BMP-4 concentrations were measured using enzyme-linked immunosorbent assay. We also measured body mass index and erythrocyte sedimentation rate (ESR), white blood cells (WBC) and neutrophil lymphocyte ratio (NLR). Mean BMP-4 levels were significantly lower in OA women compared to controls (29,66±13,61 vs 72,81±44,06 ng/ml, $P < 0.001$). Mean PRGN levels were found to be significantly lower in OA women compared to controls (71,93±33,83 vs 268,33±180,45 ng/ml, $P < 0.001$). There were no significant differences in WBC and NLR levels between two groups ($P = 0.763$, $P = 0.925$, respectively). ESR values was significantly higher in patients group than controls group ($P = 0{,}022$). There was a significant positive correlation between serum BMP-4 levels and serum PRGN levels in patients with OA. In conclusion, BMP-4 and PRGN levels may play a role in the pathogenesis of OA and could be a useful biomarker of OA.

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P14

Hematologic indices and osteoarthritis

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Ostearthritis (OA) is most common form of joint disease and one of the leading causes of disability and pain in elderly people worldwide. Historically, OA has been considered a non-inflammatory disease, but more recently studies revealed that inflammation is a risk factor associated with both progression of cartilage destruction and signs and symptoms of disease. Mean platelet volume (MPV), red blood cell distribution width (RDW), platelet distribution width (PDW), neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR) and RDW-to-platelet ratio (RPR) can be obtained with a basic hemogram test. These parameters have been investigated as a predictor of inflammatory process in many diseases, their roles in OA is unclear. The aim of the present study was to investigate the diagnostic value of routine hematological parameters on OA and explore their clinical significance. The study included 118 patients with osteoarthritis and 145 age and gender matched healthy individuals. Medical records, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), White Blood Cell (WBC) count, NLR, platelet count (PLT), PDW, RDW, RPR, PLR and MPV levels were retrospectively recorded from patient files. There were no significant differences in WBC, RDW, PLT, RPR levels between two groups (all $P > 0{,}05$). NLR and PLR values were significantly higher in OA group compared to control group (all $p < 0.001$). RBC, MPV and PDW values were significantly lower in OA than control group (all $P < 0.001$). Patients with OA had significantly higher CRP and ESR values compared to control group ($P = 0{,}020$ and $P = 0{,}011$, respectively). In addition, ESR was positively correlated with CRP and negatively correlated with RBC, MPV and PDW levels in OA patients. Also, CRP was negatively correlated with RBC and MPV levels in OA patients. Our study showed that hematological inflammatory markers might be useful parameters that can be used in patients with OA.

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P15

Serum osteopontin and bone sialoprotein levels in patients with tendinopathy

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The pathogenesis of tendinopathy remains unclear. Small integrin-binding ligand N-linked glycoproteins, a family of non-collagenous proteins including osteopontin (OPN) and bone sialoprotein (BSP), were initially thought to be limited to mineralized tissue but recent studies showed that they are more widely distributed and are expressed in nonmineralized tissues. Musculoskeletal tissue cells are categorized as the same functional unit developed from the mesenchymal stem cells. This theoretical background led us to hypothesize that OPN and BSP could be involved tendinopathy pathogenesis. The aim of this study was to investigate relationship between serum OPN and BSP levels and tendinopathy disease. Thirty-nine female tendinopathy patients and 39 female healthy volunteers were recruited for this prospective observational study. Serum OPN and BSP levels were measured using enzyme-linked immunosorbent assay. We also measured body mass index and erythrocyte sedimentation rate (ESR), white blood cells (WBC) and neutrophil lymphocyte ratio (NLR). There was no significant differences in serum BSP levels between two groups (41,83 $\pm$ 52,03 vs. 53,64 $\pm$ 53,06 ng/ml, $P=0.276$). There was also no significant differences in serum OPN levels between two groups (57,37 $\pm$ 21,61 vs. 77,72 $\pm$ 72,14 ng/ml, $P=0{,}363$ respectively). There were no significant differences in WBC, NLR and ESR values between two groups ($P=0{,}897$, $P=0.795$, $P=0{,}405$ respectively). There was no correlation between serum BSP levels and OPN, WBC, NLR and ESR levels in patients group. Patients with tendinopathy had a negative correlation between serum OPN levels and NLR levels. The results of this study have indicated that BSP and OPN levels are not involved in pathogenesis of tendinopathy.

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P16

Evaluation of hematologic parameters in patients with tendinopathy

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Tendinopathy is a painful condition that occurs in and around tendons in response to overuse. The role of inflammation and inflammatory mediators in the development or progression of tendinopathy have been investigated in many studies, but it is still uncertain and controversial. Mean platelet volume (MPV), red blood cell distribution width (RDW), platelet distribution width (PDW), neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR) and RDW-to-platelet ratio (RPR) may indicate the systemic inflammatory response associated with many diseases. In this retrospective study, we aimed to evaluate the relationship between routine hematological indices and tendinopathy. The study consisted of 65 tendinopathy patients and 77 age and gender matched healthy individuals. Age, sex, white blood cell (WBC) count, NLR, platelet count, MPV, PDW, RDW, RPR, PLR, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) were retrospectively recorded from patient files. There were no significant differences in WBC, RDW, RPR and CRP levels between two groups (all $P > 0{,}05$). Platelet values, NLR levels and PLR values of patients with tendinopathy were significantly higher than control subjects ($P=0{,}046$, $P=0.009$, $P=0.008$ respectively). MPV values, RBC values and PDW values were found to be significantly lower in patients group (all $P<0.001$). Patients with tendinopathy had higher ESR values compared to control group ($P=0{,}046$). Furthermore, ESR was positively correlated with CRP, PLT and PCT and negatively correlated with RDW and RPR in tendinopathy patients. Also, CRP was positively correlated with WBC and PLT and negatively correlated with RDW, MPV, PDW and RPR in tendinopathy patients. Results of our study have shown that hematologic parameters can be useful prognostic biomarkers in tendinopathy. Further large-scale prospective studies are required to confirm these findings and demonstrate the prognostic significance of these values.

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P17

Effects of subcutaneous administration of caffeine on bone markers in rats

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There is some evidence that caffeine consumption has effects on bone mineral metabolism, however the reports in this regard show some ambiguity. Being this compound one of the most popular for human consumption in the world, we analyzed the effects of soluble caffeine subcutaneous administration on serum osteocalcin and serum bone alkaline phosphatase (bALP) in Wistar male rats (weighting 200–250 g). Variable doses were used (30, 75, 150 mg/kg), during 30 days. The animals were kept in 12:12 h light-dark cycle with controlled temperature (22 °C) and humidity (25%). They were split into four groups ($n=4$/group): one control and three receiving the treatments with the different doses. The administration was performed 1 h after the light phase started. At the end of the experiment, blood samples were obtained by terminal cardiac puncture under anesthesia. Osteocalcin determination was performed using a specific immunoassay involving monoclonal antibodies, and bone alkaline phosphatase was determined through a heat inactivation kinetic assay. The Institutional Ethics Committee approved this protocol.

The results showed that those animals which received 30 and 75 mg/kg had significant higher levels of osteocalcin (ANOVA, $P=0.004$ y $P=0.016$, respectively) compared to those receiving 0 and 150 mg. Regarding bALP, those animals which did not receive caffeine (0 mg/kg) had increased levels of the enzyme ($P=0.015$) overall. These observations support the notion that caffeine may modulate bone remodeling depending on the dose. Prospective studies are necessary to better understand the significance of our results and the differences in effects on osteogenic potential when caffeine is administered *in vivo*.

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P18

Atorvastatin effects on a glucocorticoid-induced osteoporosis animal model

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Osteoporosis is a chronic disease with a high prevalence in older adults. There is evidence that statins have some beneficial effects on bone metabolism. Although the trial reports are ambiguous, it is now accepted that statins block the osteoclast differentiation inhibiting to some extent bone resorption. Our aim was to evaluate the effects of a statin in an animal model, which was induced to osteoporosis by glucocorticoids and then treated with atorvastatin. The experimental designed consisted of four groups: two senile osteoporotic groups (treated and untreated) and two young osteoporotic groups (treated and untreated). Osteoporosis was induced using dexamethasone (7.8 mg/kg per day) for 6 weeks. Atorvastatin treatment was administered orally at a dose of 40 mg/kg per day during 6 weeks. At the end of the experiment, animals were euthanized. Blood was obtained by terminal cardiac puncture and long bones were extracted, dehydrated and embedded in paraffin after which sections were cut on a rotary microtome. Blood determinations included osteocalcin, acid phosphatase, alkaline phosphatase, glucose, cholesterol and triglycerides using standard available kits for those biomarkers. The histological analysis was performed under light microscope and images were processed with open software Image J. The Institutional Ethics Committee approved the whole protocol.

The results showed that treatments induced reduction in serum cholesterol, confirming the effect of the statin ($P=0.04$), but the others markers did not show any significant differences. Nonetheless, the senile and young treated groups had significant higher optical densities reported by Image J that those untreated ($P=0.033$ for senile and $P=0.007$ young groups).

These results are interesting in terms of potential effect of statins, and there is evidence from a number of from basic science and clinical research studies indicating that statins may be effective treatments for osteoporosis, however more studies are under way to analyze the efficient dose to use in humans.

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P19

A novel highly specific ELISA allows the measurement of human periostin in plasma and serum

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Periostin (osteoblast-specific factor OSF-2) is a glycoprotein that in humans is encoded by the POSTN gene. It is a component of the extracellular matrix and is thought to be involved in osteoblast recruitment, attachment and spreading.
The N-terminus part of periostin is conserved, while the C-terminal region gives rise to different splice isoforms upon alternative splicing. The isoforms have a molecular weight range from 83 to 93 kDa.
We have developed a specific and sensitive sandwich ELISA for the detection of human periostin.
The assay utilizes monoclonal and purified polyclonal antibodies and recognizes epitopes that are conserved between periostin of human, mouse, rat, cynomolgus macaque, dog and cat origin. The assay is optimized for human serum and plasma (citrate, heparin, EDTA) samples. It is able to detect all known splicing forms of human periostin.
Samples from apparently healthy individuals measure within calibration point 2 and 5 out of seven points of the calibration curve, with a range of up to 4,000 pM. The detection limit of the assay is below 1 pM.
Assay characteristics, such as intra/inter-assay precision, dilution linearity and spike/recovery as well as sample stability meet standards of acceptance.
This novel ELISA provides a reliable and accurate tool for the quantitative determination of periostin in human samples.

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P20

Frozen for 7 years: How long can bones be stored prior to biomechanical testing?

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Biomechanical strength testing of bones, considered a key component in bone quality assessment, is a critical end-point in the evaluation of safety and efficacy of test compounds in preclinical studies. Bones are usually preserved frozen and tested within a few months following harvesting. They can also be stored for longer periods and only tested when additional information is required. The objective of this study was to evaluate if differences in biomechanics data between different specimens are maintained by long term storage using vertebrae stored at −20 °C from cynomolgus monkeys (cynos) 5 months after harvesting (L3 and L4) relative to 7.5 years after harvesting (T12). Effect of long term storage on absolute strength parameters was not evaluated as destructive tests were performed. L3, L4 and T12 vertebral specimens were prepared from cynos which underwent either sham or ovariectomy (OVX) surgery 17 months earlier: Sham ($n=17$) and OVX ($n=20$). Specimens were tested in compression to failure and peak load, yield load, stiffness and AUC reported. Correlation analysis of each parameter was performed between L3 and T12 tested more than 7 years apart. Correlation analyses between L3 and L4 both tested 5 months following collection were performed to evaluate the correlations between two different vertebrae stored for the same duration.

Table 1 Mean (s.d.) biomechanical parameters and correlation coefficient.

	L3		L4			T12		
	Sham	OVX	Sham	OVX	Correlation with L3	Sham	OVX	Correlation with L3
Peak Load (N)	2741±808	2329±649	2711±991	2172±628	**r=0.71**	2669±830	2550±808	**r=0.84**
Yield Load (N)	2655±816	2235±623	2622±1015	2052±658	**r=0.69**	2412±797	2159±635	**r=0.89**
Stiffness (N/mm)	12 878±4552	10 781±3353	15 736±4519	13 211±5417	r=0.48	16 658±4603	14 036±4479	r=0.43
AUC (N-mm)	1134±416	907±466	769±395	703±308	**r=0.59**	1223±661	1084±677	**r=0.69**

$P<0.0001$.

For L3, L4 and T12, group means and s.d. were comparable in sham and OVX groups, respectively, Table 1. Correlation analysis showed significant positive linear relationships between peak load, yield load or AUC between L3 and T12, Table 1 ($r=0.69$ to 0.89). Correlation between L3 and L4 tested after the same storage duration were comparable.
Although the absolute preservation of bone strength with long term storage cannot be confirmed with destructive tests, the strong correlation of biomechanical properties of different specimens before and after storage, support the validity of long-term preservation of specimens for future biomechanical testing.

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Profiles of 25 hydroxyvitamin D and its metabolites 24, 25-dihydroxyvitamin D and 1, 25-dihydroxyvitamin D in vitamin D_3 supplementation studies

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Background
Vitamin D plays an important role in calcium and phosphate homeostasis. Circulating 25 hydroxyvitamin D [25(OH)D] is metabolised into its biologically active form 1,25-dihydroxyvitamin D [1,25-d(OH)D] by the actions of 1α hydroxylase, and into inactive 2metabolite 24,25-dihydroxyvitamin D [24,25-d(OH)D] by 24-hydroxylase. Recent studies suggest the production of 1,25-d(OH)D) from 25(OH)D is 24,25-d(OH)D dependent. Genetic mutations of CYP24A1 gene resulting in reduced or total loss of 24-hydroxylase function are associated with hypercalcaemic conditions.
Objective
To establish the profiles of 25(OH)D, 24,25-d(OH)D and 1,25-d(OH)D in non-supplemented healthy individuals and investigate changes in three vitamin D_3 supplementation studies.
Method
Samples obtained from a group of non-supplemented individuals and three vitamin D_3 supplement studies (Table 1) were measured for 25(OH)D_3/D_2 and 24,25-d(OH)D_3/D_2 and total 1,25-d(OH)D.
Results
Mean 25(OH)D:24,25-d(OH)D ratio was established from non-supplemented subjects (Table 1). Subjects supplemented with daily vitamin D_3 showed rapid increase in 25(OH)D and 24,25-d(OH)D concentrations, whereas only a moderate increase in 1,25-d(OH)D concentration. No significant changes were observed in 25(OH)D:24,25-d(OH)D ratio in subjects given more than 5000 IU, a decrease in ratio was found in subject given 1000 IU/day or less.
Conclusion
Increasing vitamin D_3 supplementation results in an increase in metabolites but relative difference in production of 1,25-d(OH)D. Our results indicate that the metabolism favors the production of 24,25-d(OH)D than of 1,25-d(OH)D in the presence of high supplementation of vitamin D_3 in contrast to regular low dosage in order to prevent toxicity. We advise caution when using extremely high supplementation levels may not be beneficial to the patients.

Table 1

		Mean±s.d. 25(OH)D nmol/l		Mean±s.d. 24,25-d(OH)D nmol/l		Mean±s.d. 1,25–d(OH)D pmol/l		Mean±s.d. 25(OH)D:24,25-d(OH) D ratio		Mean±s.d. 1,25-d(OH)D:24,25-d(OH)D ratios	
n	Vitamin D dose (IU)	Baseline	Peak	Baseline	Peak	Baseline	Peak	Baseline	Peak	Baseline	Peak
474	None	63.1±24.2	–	5.6±2.9	–	–	–	13±4	–	–	–
69	Single 100,000	33.4±11.7	80.2±20.8	2.4±1.0	6.8±2.0	99.1±27.4	125.1±42.2	15±3	14±4	50±24	20±8
43	5000 and 10,000 daily	86.3±19.3	176±58.8	7.9±2.2	14.7±4.5	104.8±44.2	135.3±56.2	12±3	13±4	15±6	10±4
253	400 and 1000 daily	44.5±18.2	75±24.2	2.8±1.6	6.2±2.8	–	–	16±4	13±3	–	–

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Bone biomechanics and quality

P22

Finite element analyses predict the mechanical impact of cam-FAI surgery on ovine femurs

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Cam femoro-acetabular impingement (FAI) surgery restores hip range of motion by trimming the head–neck junction, which weakens the proximal femur. Sheep femurs resemble cam deformity and might be suitable for evaluating the impact of the surgical correction. Accordingly, this study addressed two questions: How does increasing head-neck resection affect ovine femoral strength? Are quantitative computed tomography (QCT)-based FE models able to discriminate the observed changes?

With approval of the veterinary board (Kantonales Veterinäramt Zürich, application 123/2006), 18 femoral pairs were distributed in three groups (3, 6 and 9 mm resection depth) and one random bone of each pair underwent surgery. Then, specimen-specific models were generated for each pair from QCT scans performed with a calibration phantom. Finally, compression tests were conducted *ex vivo* and *in silico* under stance configuration and experimental and simulated failure loads were quantified. Safety factors (SF) were calculated for walking and running activities based on the BMI. The weakening of the resected femurs was evaluated relative to their intact contralateral side.

Our results showed that *ex vivo* and *in silico* failure loads correlated strongly ($r^2 = 0.83$, $P < 0.001$) and exceeded hip forces induced during daily activities, even after strong resection (SF > 1). For each group, the resections had more influence on the FE failure loads (−18%, −21%, −33%) than measured experimentally (−5%, −10%, −19%). The differences between resection groups were significant or close to $P = 0.05$.

Two conclusions can be drawn from these results. First, just as for human hip, strength of ovine femurs significantly decreases with deeper resections. Fracture risk induced by the procedure, however, remains low even after 9 mm correction. Second, this study yielded first evidence of QCT-based models predicting the weakening of femurs after head–neck resection. Based on pre-operative CT, those models could provide patient-specific guidelines to prevent over-correction during cam FAI surgery.

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Impact of visceral adiposity on trabecular bone score in obese postmenopausal women: A cohort study

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Background

Higher BMI values are associated with greater bone mineral density (BMD) resulting in a site-specific protective effect for fragility fractures. However, pathophysiologic influence of central adiposity on bone quality may oppose the seemingly positive influence of a greater mechanical loading with higher body masses. The objective of our study was to evaluate the impact of visceral fat on trabecular bone microarchitecture in postmenopausal obese women.

Materials and methods

In this study we reported data from medical records of obese postmenopausal women (BMI $\geqq 30$ kg/m^2). In this population we assessed BMD at lumbar spine (LS BMD) and at femoral neck (FN BMD), Trabecular Bone Score (TBS), VAT volume and VAT mass. We divided our population into quartiles of VAT volume. SPSS 21.0 was used to assess the differences between groups in TBS, according to cut-off proposed by Silva *et al.* [1].

Results

Data of 226 women were reported in table 1.

Conclusions

Our findings suggest that visceral fat might play a key role in bone microarchitectural changes in obese women. TBS might be used to identify obese individuals at highest risk of impaired bone strength. However, additional researches to identify mechanisms linking greater adiposity to adverse effects on bone are needed.

Table 1

	Total (n=226)	VAT < 1,398 cm^3 (n=56)	VAT 1,398 < x < 1,764 cm^3 (n=57)	VAT 1,765 < x < 2,371 cm^3 (n=57)	VAT > 2,371 cm^3 (n=56)	P value
Age (years)	64.56 ± 8.42	63.26 ± 8.87	63.86 ± 7.90	65.25 ± 9.11	65.87 ± 7.57	0.332*
BMI (kg/m^2)	34.18 ± 3.32	33.00 ± 2.26	33.58 ± 2.80	34.34 ± 2.83	35.83 ± 4.42	<0.001*
TBS	1.17 ± 0.14	1.23 ± 0.14	1.18 ± 0.15	1.17 ± 0.12	1.11 ± 0.13	<0.001*
LS BMD	1.091 ± 0.18	1.083 ± 0.17	1,101 ± 0.20	1.086 ± 0,17	1.093 ± 0.17	0.984*
LS T-score	−0.715 ± 1.53	−0.79 ± 1.49	−0.56 ± 1.73	−0.78 ± 1.44	−0.70 ± 1.46	0.982*

*Kruskal–Wallis test for independent samples.

Reference

1. Silva BC, *et al.* "Trabecular bone score: a noninvasive analytical method based upon the DXA image". *J Bone Miner Res* 2014; Mar 29(3): 518–30.

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P24

Epidemiological and biomechanical influences on the prevalence and progression of periprosthetic osteolisis after total hip replacement: analysis with magnetic resonance imaging

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Introduction and objectives

There is an increasing interest in knowing the factors related to the wear particle-induced disease in total hip replacement (THR), after long-term follow up. Numerous studies have analyzed the development and the destruction patterns of the osteolisis. The objectives of this study were to determine the factors associated with major frequency and severity of this disease and their influence in the osteolytic progression pattern, using magnetic resonance imaging (MRI)

Methods

Our study included 86 consecutive THR of the same model, with a circunferencial porous hydroxyapatite-coating, implanted between 1990 and 2007. The mean follow up were 13, 20 years. We performed a clinical and radiological analysis recording epidemiological and biomechanical variables including the polyethylene (PE) wear, using a specific software. In each case a MRI was performed, applying special protocols in order to reduce artifacts. We evaluated the location, size and osteolytic progression pattern. Finally, we made a statistical analysis. Pearson correlation and multiple regression techniques were used to analyze the data.

Results

We found statistically significant differences ($P < 0.05$) between the osteolytic size and many variables: patients age at primary surgery ($r = -0.239$), physical activity ($r = 0.325$), acetabular inclination ($r = 0.231$) and rate of PE wear ($r = 0.484$).

Conclusions

The severity of osteolytic damages is larger in young patients, with more postsurgical physical activity. These factors, as well as acetabular inclination, are associated with increased PE wear rate and larger progression of the osteolytic disease. Higher wear rate and large osteolytic lesions were related with peripheral and continuous destruction patterns.

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P25

Effect of paracetamol on bone mechanical properties in rats

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Results of epidemiological studies indicate that paracetamol use may be a risk factor for fracture. The exact mechanism of paracetamol action remains unclear,

but it is known to inhibit COX-2. Orchidectomy-induced androgen deficiency was reported to increase COX-2 expression and prostanoid release in rats. The aim of the present study was to investigate the effect of paracetamol on bone mechanical properties in rats with normal androgen levels and with androgen deficiency induced by orchidectomy.
Three-month-old Wistar rats were orchidectomized (ORX) or sham-operated (Sham), and, after 7 days, divided into six groups ($n=10$): sham-operated control rats, sham-operated rats receiving paracetamol (70 or 210 mg/kg), ORX control rats, ORX rats receiving paracetamol (70 or 210 mg/kg). Paracetamol was administered by a gastric tube once daily, for 7 weeks (6 days a week). Mechanical properties of the proximal tibial metaphysis, femoral diaphysis and femoral neck, as well as histomorphometric parameters: transverse cross-section area of the cortical bone and width of trabeculae in the distal epiphysis and metaphysis in the femur were determined 8 weeks after ORX or sham operation. The paracetamol effect on bone strength was dose- and androgen level-dependent. Paracetamol at 70 mg/kg did not significantly affect the skeletal system in Sham rats and tended to unfavorably affect it in ORX rats. At 210 mg/kg, paracetamol non-significantly worsened mechanical properties of cancellous bone (the tibial metaphysis) in Sham rats, whereas it counteracted the orchidectomy-induced worsening of histomorphometric parameters and mechanical properties of both cancellous and compact bone (the tibial metaphysis, and femoral diaphysis and neck) in ORX rats.
In conclusion, long-term use of high-dose paracetamol (210 mg/kg for 7 weeks) favorably affected the skeletal system of androgen-deficient rats.

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Doxazosin prevents the development of estrogen deficiency-induced osteoporosis in rats

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In postmenopausal women, estrogen deficiency not only interferes with the metabolism of the bone tissue, but also adversely affects the cardiovascular system and can cause hypertension. Doxazosin is often used in the treatment of resistant hypertension, especially in patients with renal failure and hyperlipidemia. Vasodilator effect of doxazosin is associated with selective blocking of $\alpha 1$ receptors in the smooth muscle cells of resistance arterioles. The results of epidemiological studies on the effects of $\alpha 1$-blockers on the skeletal system are contradictory and no studies on the effect of doxazosin on the development of experimental estrogen deficiency-induced osteoporosis have been reported. The aim of this study was to investigate the effect of doxazosin on bone remodeling and mechanical properties of the femur in ovariectomized rats.
The experiments were performed on 3-month-old female Wistar rats, divided into four groups ($n=9$–10): sham-operated control rats, ovariectomized (OVX) control rats, OVX rats treated with doxazosin 0.5 or 5 mg/kg p.o. daily for 4 weeks. The bilateral ovariectomy was performed 7 days before the start of doxazosin administration. Femoral bone mass, mineralization, histomorphometric parameters (periosteal and endosteal transverse growth, cortical bone and marrow cavity area in the diaphysis, and trabecular width in the epiphysis and metaphysis) and mechanical properties of the diaphysis and neck were investigated.
Estrogen deficiency induced osteoporosis with intensification of bone resorption and formation. Doxazosin dose-dependently counteracted the development of osteoporosis, increasing bone formation and/or inhibiting bone resorption in cancellous and compact bone. Moreover, doxazosin improved mechanical properties of compact bone (the femoral diaphysis).
In conclusion, the results of this experimental study using an animal model of osteoporosis, suggest that doxazosin used in the treatment of hypertension may decrease the risk of osteoporosis in postmenopausal women.

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P27

Relationships between morphometric, densitometric and mechanical properties of tibiotarsal bone in 14-month-old ostriches (*Struthio Camelus*)

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The aim of the study was to evaluate relationships between morphometric, densitometric and mechanical properties of tibiotarsus in ostriches (*Struthio camelus*). 14-month-old males ($N=15$) and females ($N=31$) were slaughter to obtain left tibiotarsus. Using computed tomography technique (Somatom Emotion scaner, Siemens), volumetric bone mineral density (vBMD) of the trabecular (Td) and cortical bone (Cd), mean voulmetric bone mineral density (MvBMD) and cortical bone area (CBA) were measured. Calcium hydroxyapatite density of the trabecular bone (TbCa-HA) and cortical bone (CbCa-HA) were determined using 10-mm thick epiphyseal and middiaphyseal scans and Osteo CT application package. Bone mineral density (BMD) and bone mineral content (BMC) were evaluated using dual-energy X-ray absorptiometry (DEXA) method. Using three-point bending test (INSTRON 3367 apparatus, Instron, USA), ultimate strength (Wf) of tibiotarsus was determined. Pearson's correlation coefficient (r) was determined between all the investigated variables. $P<0.05$ was statistically significant. Positive correlations of the final body weight with bone weight and BMC of tibiotarsus were found, while body weight was negatively correlated with relative bone weight (RBW) ($P<0.05$). Bone length was positively correlated with bone weigth, RBW and BMC, while bone weight was positively correlated with RBW, BMC, CBA and Wf ($P<0.05$). BMD was positively correlated with BMC, CBA, MvBMD, Td and Wf, while BMC was positively correlated with CBA and Wf ($P<0.05$). CBA was negatively correlated with Cd and positively correlated with Wf ($P<0.05$). MvBMD was positively correlated with Cd, CbCa-HA and Wf, while Cd was positively correlated with Td, CbCa-HA and TbCa-HA ($P<0.05$). Positive correlation of Td and TbCa-HA was found ($P<0.05$).
In conclusion, this study showed numerous interrelationships between morphometric, densitometric and mechanical properties of tibiotarsus in ostrich. Tibiotarsus may be used as an attractive experimental model for further studies on metabolic response of skeleton to physiological, nutritional, toxicological and pharmacological factors influencing bone tissue metabolism.

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P28

Alendronate therapy improves anterior vertebral microstructure in osteoporotic bone facilitating fracture risk reduction

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Although the fracture risk reduction under bisphosphonate treatment in osteoporosis is clinically well established, it remains understudied why the efficacy of bisphosphonate therapy is higher for the prevention of vertebral fractures compared to other fractures.
Our aim was to investigate whether anti-resorptive therapy with alendronate would result in different regional variations of structural indices in the vertebral body. We investigated the microstructure of L3 vertebrae from 14 women (mean age 83 years) with high-resolution peripheral quantitative CT (82 µm). Further, we scanned cylindrical samples from the anterior and posterior vertebrae with micro-computed tomography at 3.5 µm resolution and determined their mechanical competence with uniaxial compression testing.
The CT results showed a significantly higher average bone volume to tissue volume ratio (BV/TV), T-Score, and trabecular thickness for the alendronate treated group compared to the osteoporotic control group. Regression analysis showed a high influence of BV/TV on the maximum force for both groups.

While our results for the regional structural indices in the osteoporosis group agree well with epidemiological data, we found specific deviations in regional structures for the alendronate group. The osteoporosis group had a significantly lower BV/TV and trabecular number in the anterior regions compared to the posterior ones. In contrast, the alendronate group did not exhibit these regional differences. Further, trabecular thickness was higher for the anterior cylinders from the alendronate group than those from the osteoporosis group.
Alendronate therapy influences the regional variation (anterior vs posterior) of vertebral BV/TV in comparison to treatment naïve controls. Contrary to osteoporotic bone this study shows a uniform distribution of bone volume in anterior and posterior regions for alendronate treated bone. This uniform distribution might make the elderly vertebrae less susceptible to wedge fractures. Therefore, our study provides insight in the higher fracture risk reduction in vertebrae compared with other fracture sites.

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P29

Modified stable supracondylar corrective osteotomy technique for Genu Valgum

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Introduction
Genu valgum is a common deformity in Indian (aediatric population owing to nutritional and social reasons), and is often neglected. The patient, especially females, present at around marriageable age demanding a single stage, cosmetic, implantless correction of deformity.
Objectives
To present a technique of modified supracondylar osteotomy for correction of genu valgum deformity and evaluation of results.
Materials and method
Eleven knees in seven patients underwent a varus distal femoral osteotomy via medial approach followed by a stable deformity correction and cast application. No implants were used. Patients were followed up clinically and radiographically and mobilised within the pain limits and cast removed at 6 weeks. The duration of follow up was 12 months.
Results
All patients returned to preoperative range of motion at 3 months, excellent correction of deformity, with a well acceptable minimal medial scar, no infection and complete union of osteotomy site. All patients were highly satisfied with their results.
Conclusion
In our case series we found this procedure as an effective method to correct the valgus deformity. Advantages are complete correction of deformity, low morbidity, cost effectiveness, good stability, no need for internal fixation, single stage procedure, rapid healing and possibility to readjust alignment post-operatively, well acceptable scar and full return of range of motion.

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P30

Chemical composition of the trabecular bone with and without osteoarthritis

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Purpose
The aim of this study is to determine the chemical information in trabecular bone by Raman spectroscopy in the human distal femur at the microstructural level.
Methods
The subchondral trabeculae were obtained from the middle of medial articular surface of the distal femurs in the two cadavers (one is with and the other is without OA). Raman spectroscopy, a non-destructive technique, was employed to determine the chemical information of the trabecular bones in the distal femurs.
Results
The maximum intensity of the phosphate peak observed between the two groups (The cadavers, one is with and the other is without OA) was significantly different ($P=0.017$). The maximum intensity of the amide I peak was significantly different between the two groups ($P=0.042$). Also, among the other chemical and matrix components (Hydroxyproline, Carbonate, Amide III, and CH2), the spectrums showed similar significant differences in the intensity ($P=0.027$, $P=0.014$, $P=0.012$, $P=0.029$). The area integration were performed to determine disorder in collagen's secondary structure via amide III (alpha helix/random coil). The value of the alpha helix to random coil band area was significantly different. So the result shows that there was a trend toward higher collagen maturity for the non-OA bone specimens.
Conclusions
The result suggested that OA might affect the chemical compositions of trabecular bone, and such distinctive chemical information.

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P31

Site specific bone loss induced by spaceflight in mice: a multiscale evaluation of trabecular and cortical structure and quality

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Intrinsic quality, micro and nano structure of bone after 1 month of spaceflight and during 1 week of recovery had never been investigated. The aim was to explore these properties in appendicular and axial skeleton of mature mice.
Ten C57/Bl6 male mice flew on the 30-day space Russian BION-M1 high-orbit satellite mission (Biomedical Ethics Commission of IBMP, n°319). Five were euthanized 12 h after landing, the others 8 days later. A ground control group was kept in the same conditions as in flight (housing, food and climate).
Body weight, soleus muscle mass, and femur length were not significantly different between groups.
In the femur, microgravity significantly decreased trabecular BV/TV (−65% vs Ctr) (micro-CT), increased trabecular and periosteal TRAP+ osteoclast surfaces and marrow adiposity (X22 vs Ctr), and decreased Ct.Th (−5.4% vs Ctr). Nano-indentation analysis indicated that cortical modulus, hardness and working energy were significantly decreased whereas the degree of mineralization (micro radiography) was not affected by microgravity. Microgravity did not affect cortical osteocyte lacuna density but decreased their mean 3D volume (Synchrotron Radiation nanotomography, $n>8000$ lacunae per sample). Eight days after landing, osteoclast cell surface were restored but trabecular BV/TV and Ct.Th remained lower than Ctr group.
In vertebrae, microgravity significantly decreased BV/TV (−36% vs Ctr) and the modulus in the younger mineralized matrix which tended to increase after recovery. Degree of mineralization, mineral and collagen intrinsic quality (infra-red spectroscopy) were not changed by microgravity.
To summarize, microgravity severely decreased bone mass, altered bone structure and intrinsic mechanical properties in both sites and increased femoral marrow adiposity. One week after landing, these properties tended to be rescued in vertebrae but remained altered in the femur despite normalized osteoclastic cell surfaces. The decrease of osteocyte lacunar volume suggests the existence of an intra-cortical bone preservation mechanism in absence of mechanical loading.

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Characterization of bone using ultrasonic waves and structural borne acoustic waves

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An orthogonally-anisotropic Biot-Johnson-Allard (BJA) model in which the dependences of tortuosity on porosity and angle are determined empirically from acoustic measurements on bone replicas has been developed. Phase velocities and attenuations of the fast and slow waves versus frequency, porosity and angle of propagation have been predicted by using BJA model. The attenuation of the fast wave is below 0.5 Np/m throughout the frequency and propagation angle range. The attenuation of the slow wave is around 1.7 Np/m throughout the frequency and propagation angle range. We also investigated the use of structural borne acoustic wave technique to diagnose the osteoporosis. When normal and

osteoporotic bones are subjected to vibration, the resulting detected responses have different shapes, different natural frequencies, and amplitudes. Differences between normal and osteoporotic bones might be the sign of osteoporosis being diagnosed by structural vibration technique.

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Assessment of changes in collagen associated with advanced glycation end-products in human bone using vibrational spectroscopy

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Introduction
Aging, diabetes and other disease conditions are associated with the accumulation of non-enzymatic collagen cross-links (NE-XL) in human tissues. Non-enzymatic cross-links (i.e. advanced glycation end products (AGEs)), occur at the bone collagen level, where they are associated with individuals increased fracture risk caused by bone's reduced plasticity.
Methods
Here, non-destructive Fourier-transform infrared (FTIR) spectroscopy was used to investigate the two-dimensional spatial distribution of NE-XL in the bone matrix. A cohort of human cortical bone that had been control and ribose-treated to increase the AGE cross-link content *in vitro* were used to determine the correspondence between the area ratio of the 1678/1692 cm^{-1} subbands and an increase in AGEs. The method was validated on a specific cohort of bone cores acquired before and after bisphosphonate treatment, which is associated with *in vivo* AGE accumulation.
Results
The peak area ratio of the amide I subbands at 1678 and 1692 cm^{-1} shows changes with the increase in AGEs (control: 3.79 ± 0.36, ribation: 4.31 ± 0.45, $P < 0.05$) and its measurement does not require demineralization of the sample. Using this new approach, significant increases in the AGE cross-link ratio were measured in iliac crest biopsies after bisphosphonate treatment (pre-treatment: 3.57 ± 0.19, post-treatment: 3.88 ± 0.26, $P < 0.05$), which is known to increase *in vivo* AGE cross-links.
Discussion
The method provides a direct quantitative measure of collagen quality in bone. The new defined non-enzymatic cross-link ratio (NE-XLR), allowing a spatial quantitative assessment of AGE cross-links using FTIR, will be highly valuable to investigations of bone quality in cases of aging, disease and treatments.
Clearly, identified area ratio of the amide-I subbands (NE-XLR) are primed for high spatial resolution measures of collagen quality (6.25 μm), which is of crucial importance to understand the role of collagenous cross-links in bone's mechanical competence.

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Abstract withdrawn.

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Bone material properties as measured by Reference Point Indentation are low in subjects with acromegaly

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Introduction
Acromegaly is a rare disease caused by excess growth hormone (GH) production by an adenoma of the anterior pituitary gland. The skeletal complications of GH and IGF-1 excess include increased bone turnover, increased cortical bone mass and deteriorated microarchitecture of trabecular bone, associated with a high risk of vertebral fractures in the presence of a relatively normal Bone Mineral Density (BMD). There are no data on bone material properties in patients with acromegaly.
Objective
To evaluate bone material properties in patients with acromegaly using Reference Point Indentation (RPI).
Methods
Cross-sectional study in 32 well-controlled acromegaly patients aged ≥ 18 years compared to 32 age-and fracture-matched controls. Bone Material Strength index(BMSi) was measured by RPI using the Osteoprobe. Two independent operators assessed vertebral fractures using the semi-quantitative method of Genant.
Results
Mean age of acromegaly patients (53% male) was 56.6 years (range 37.9–76.5), and 56.4 years (range 35.2–77.2) in controls (41% male). Patients with acromegaly had higher BMI ($28.2 \text{ kg/m}^2 \pm 0.9$ vs $24.0 \text{ kg/m}^2 \pm 0.6$, $P < 0.001$) and higher BMD at lumbar spine ($1.06 \text{ g/cm}^2 \pm 0.20$ vs. $0.92 \text{ g/cm}^2 \pm 0.17$, $P = 0.006$) and femoral neck ($0.85 \text{ g/cm}^2 \pm 0.15$ vs. $0.73 \text{ g/cm}^2 \pm 0.11$, $P < 0.001$) than controls. However, adjusted for BMI, LS BMD was not significantly different between acromegaly patients and controls.
BMSi was significantly lower in acromegaly patients than in controls (79.9 ± 0.8 vs. 83.5 ± 0.8, $P = 0.004$). There was no difference in BMSi values between acromegaly patients with ($n = 13$) or without ($n = 19$) vertebral fractures (79.3 ± 1.3 vs. 79.1 ± 1.1, $P = 0.931$), nor was there a relation between morphometric vertebral fractures and BMSi in the control group.
Conclusion
Patients with acromegaly have significantly lower Bone Material Strength index compared to controls. Furthermore, BMSi was comparable in acromegaly patients, with or without vertebral fractures. Our findings suggest that material properties of bone are impaired in patients with acromegaly and suggest that other measures than BMD should be considered to evaluate bone fragility in acromegaly.

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Finite element analysis of osteoporotic lumbar vertebrae L_1 under dynamic loading

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The present study introduces in silico analysis of osteoporotic lumbar vertebrae L_1 due to dynamic compressive load.
Objective
To define the reduction of load carrying capacity caused by various grades of osteoporosis.
Materials and methods
The three-dimensional inhomogeneous continuum problem was formulated for simulation purposes. The L_1 vertebra model consists of cortical shell, trabecular network and posterior elements. Bone tissues are modelled as elastoplastic and transversally isotropic solid. External loads are transmitted via weaker elastic intervertebral disks. The influence of osteoporotic aging was modelled by reducing the thickness of cortical shell and the elasticity modulus of trabecular network. Power law describes osteoporotic degradation of elasticity properties

against density. The three-dimensional FE model was developed and the parametric study of various grades of low bone density and the reduced thickness of the cortical shell was performed. The failure load is evaluated by applying the von Mises-Hencky strength criterion.
Results
The numerical results of parametric study showed a significant dependence between the values of failure load and state of bone tissue. The failure load appears while dynamic load pressure reaches 0.3 MPa for model with 0.2 mm cortical shell width, on condition where apparent density of trabecular bone is <0.25 g/cm^3. At the same time, the model with 0.5 mm cortical shell could carry the twice higher load pressure or 0.6 MPa, while apparent density of trabecular bone was 0.10 g/cm^3. The model of healthy bone was resistant to 0.75 MPa load with the load-carrying capacity of bone about 20%, while the decrease of the thickness of cortical shell by 0.1 mm reduces the load-carrying capacity by 25%.
Conclusion
Parametric study demonstrates the decisive role of the cortical shell, the thickness of which was figured as the primary parameter of strength of the whole lumbar vertebrae body.

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Elemental composition of compact human bone correlated with the osteocyte network

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Spatial distributions of major and minor chemical elements are supposed to change during tissue maturation and due to bone diseases. Previous studies suggested that osteocytes have the ability to interact with the bone matrix of their environment. For this interaction osteocytes make use of the big inner surface of the osteocyte lacuno-canalicular network (OLCN) in which they are accommodated. The aim of this study was to quantify spatial correlations between the elemental composition of bone with the appearance of the OLCN to observe the possible impact of the network on the mineralized bone matrix.
To visualize and classify the OLCN structure, confocal laser-scanning microscopy (CLSM) was used after staining samples of human compact bone (children and adults, femoral midshaft) with rhodamine-6G. The analysis of the elemental composition of bone was performed using a scanning electron microscope equipped with an energy dispersive X-ray analysis (EDX) detector. Local concentrations of Ca, P, Mg and Na were evaluated and quantified in regions of 40×30 µm^2 selected based on differences in OLCN characteristics.
Regions of interstitial bone without any accessible OLCN showed the highest values of the Ca content (11.5–13 at %Ca). In contrast, regions of osteonal bone with an intact network had lower Ca content with values down to 8 at %Ca. While the Na concentration increased with increasing Ca this relation was not observed for Mg. Also in the comparison between children and adults these elements behaved differently with Na higher in adults and Mg higher in children. The Ca/P ratio increased slightly from 1.6 to 1.8 (theoretical value for hydroxyapatite equals 1.67) with the Ca content. Variations in the chemical compositions of human cortical bone were found depending on i) Ca content (interpretable as tissue age), ii) individual age and iii) canalicular network.

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Arthritis induces early bone structural degradation and mechanical weakness

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Background
We have previously found in the chronic SKG mouse model of arthritis that long standing (5 and 8 months) inflammation directly leads to high collagen bone turnover, disorganization of the collagen network, disturbed bone microstructure and ultimately declining in bone biomechanical properties. Our main goal was to study the effects of the inflammatory process on the microarchitecture and mechanical properties of bone in the early stages of arthritis development.
Methods
Fifty Wistar adjuvant-induced arthritis (AIA) rats were monitored throughout arthritis development and sacrificed after 4, 11 and 22 days of disease induction. Thirty healthy non-arthritic rats, age and sex-matched, were sacrificed at the end of the experiment and used as controls for comparison. The inflammatory score, ankle perimeter and body weight were measured over the experimental period. At the time of sacrifice, bone and serum samples were collected for micro-CT and three-point bending analysis as well as bone turnover markers (CTX-I and P1NP), respectively. All experiments were approved by the Animal User and Ethical Committees at the Instituto de Medicina Molecular (Lisbon University), according to the Portuguese law and the European recommendations.
Results
We have observed that bone turnover markers, CTX-I and P1NP, increased soon after arthritis onset ($P<0.0001$ and $P=0.0034$, respectively, when compared to healthy controls). Moreover, micro-CT analyses showed both in trabecular and cortical parameters, that the effects of inflammation on bone microstructure were evident since the 4th day of arthritis development. Of particular interest, trabecular bone volume fraction decreased and cortical porosity increased at day 22 post disease induction when comparing to healthy controls ($P=0.0001$ and $P<0.0001$, respectively). Biomechanical tests revealed that arthritic bone have altered biomechanical properties, such as maximal bending force (arthritic group lower than healthy control, $P<0.0001$).
Conclusions
The inflammatory process induced bone loss, and reduces bone strength since the very early phase of arthritis.

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Depth and location dependence of subchondral trabecular structure across the tibia in human osteoarthritic knee versus normal knee: a micro-CT study

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Objective
To determine differences between osteoarthritic (OA) knees with normal knees for subchondral trabecular bone structure according to depth and location in the tibial plateau.
Methods
In a population of 30 cadaveric left knees (18 women and 12 men, mean age: 79.1 years $\pm$ 8.2, range: 63–90) the Kellgren-Lawrence (KL) score was determined from post mortem radiographs: OA = KL $\geqq$ 2 ($n=6$ women, $n=5$ men) and controls = KL $\leq$ 1.
After dissection, two vertical cores (7 mm in diameter) were extracted in the central region of the medial tibial plateau (S1) not covered by meniscus and peripheral (S2) fully covered by the meniscus. Each core was imaged with micro-CT (SkyScan 1172®) pixel size 10.23 µm. In the first 1–5 mm and the 6–10 mm beneath the subchondral bone plate: Bone Volume/Total Volume (BV/TV,%), Trabecular Number (Tb.N,1/mm), Trabecular Thickness (Tb.Th,mm), Trabecular Separation (Tb.Sp,mm), Degree of Anisotropy (DA), Structure Model Index (SMI) and Trabecular Bone Factor (Tb.Pf) were measured.
To investigate differences in trabecular bone structure between OA and controls, a separate analysis of covariance with age as covariate was performed for men and women separately.
Results
At 1-5mm, in G1 location for both men and women, Tb.N, Tb.Sp, Tb.Pf and SMI were significantly different ($0.04<P<0.001$), for men BV/TV was also different ($P=0.006$) and for women DA ($P=0.01$). In G2 location for both men and women, BV/TV and Tb.N were significantly different ($0.04<P<0.02$). Tb.Sp and DA were also significantly different in women $P=0.01$ and $P=0.03$, respectively. At 6–10 mm, there was no difference of any parameters between OA and controls in both men and women.

Conclusions
We conclude that the impact of OA in subchondral bone was only present in the first 5mm beneath the subchondral plate with constantly found an increase of Tb.N, and in a lesser extend a greater connectivity, a more plate like structure and a decrease of anisotropy.

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Atypical fractures of femur after longterm bisphosphonates treatment: two years follow up

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The authors present own group of 4213 patients treated with bisphosphonates (BFs) and development of atypical fractures (AF) after 2 years follow up. At the beginning 2147 patients were treated with alendronate and 2066 with ibandronate. Totally 15 fractures were found in the alendronate group (two in adult patients with osteogenesis imperfecta) and 14 in the ibandronate group (one in adult patients with osteogenesis imperfecta). All radiographic examinations were subjected to the retrospective analysis of fracture type including fractures of femur and humerus.
Results
In the alendronate group four pertrochanteric, six diaphysis of femur, two subtrochanteric and two periprothetic fractures were identified. No fracture of femur was found in this group. Based on radiography analysis after trauma or surgery three fractures met the criteria for an atypical fracture in the alendronate group. In the ibandronate group four pertrochanteric, two subtrochanteric, five fractures of femur diaphysis and two periprosthetic fractures in patients with endoprothesis were identified. There was no fracture of femur found and only 1 fracture met the criteria for AF in the ibandronate group.
After 2 years most of the patients were actively treated, the part stopped or took a drug holiday. 2460 patients on alendronate (2166 actively treated, 294 newly initiated) and 2169 on ibandronate (1911 actively treated, 258 newly initiated) were checked for atypical fracture development with the same methodology like 2 years ago. There was only one patient with a new atypical fracture of femur identified.
The authors discuss identification of risk factors for future AF development at the beginning of therapy, especially proximal femur curvature. Atypical fractures are usually very rare when diagnosis, indication of therapy and carefull follow up is done properly.

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Investigation of the potential link between mechanosensory proteins PC1/PC2 and craniosynostosis

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Background
Skull development is a tightly regulated process that occurs along the osteogenic interfaces of the cranial sutures that allow rapid bone formation at the edges of the bone fronts. Premature closure of cranial sutures can result in pathological conditions such as Craniosynostosis. The mechanosensory proteins Polycystin 1 (PC1) and 2 (PC2) have been shown to regulate skeletal development and potentially suture formation.
Aim
The aim of study was to investigate the implication of PC1/PC2 in suture development and suture fusion.
Methods
Presence of PC1 and PC2 proteins was investigated in rat sagittal (SAG) sutures tissue sections during postnatal development at p1/p5/p15 days of Sprague Dawley rats. PC1/PC2 localization and expression levels were investigated in primary suture SAG cell populations and human craniosynostosis tissue samples by RT-PCR, PCR, Western Immunoblotting and Immunohistochemistry. Ethical approval was obtained for all experimental protocols.
Results
Western Immunoblotting revealed a differential expression pattern for PC1 and PC2 in SAG sutures at p1/p5/p15 days. PC1/PC2 levels were elevated at postnatal day 5. Immunohistochemical analysis showed nuclear localization of PC1/PC2 expression in fused sutures. In primary suture SAG cell cultures, PC1 and PC2 presence was associated with an elevated expression of the osteoblast marker RUNX2 and a lower expression of chondrocyte marker SOX-9. PC1 and PC2 expression in human craniosynostosis samples was detected in the area of synostotic sutures.
Conclusion
Polycystins are implicated in suture formation and growth, playing a potential role in premature obliteration of sutures that occur in pathological conditions such as Craniosynostosis. Further research is required to elucidate PC-induced molecular pathways in suture fusion processes for detection of specific molecular targets to complement current therapy and diagnostic schemes.

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P42

Sexually dimorphic response on bone development after neonatal thymectomy in rats

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The effects of neonatal thymectomy, at 3 days of age, on skeletal sexual dimorphism and bone quality during the development were examined in male and female Wistar rats. Rats were euthanized at 20, 40, or 120 postnatal days and femurs were collected. The Biomechanical compression test was used to evaluate bone breaking strength, energy-to-fracture, and extrinsic stiffness. During the prepubertal period, the neonatal thymectomy increases extrinsic stiffness of bone tissue significantly ($P=0.0243$) only in both groups of males. The energy-to-fracture was increased in both groups of females compared with control males, showing the sex influence ($P=0.0243$) on this variable. The bone breaking strength showed a significant interaction between treatment and sex ($P=0.0108$), neonatally-thymectomized males were higher than their respective controls and both groups of females groups. At puberty, the treatment was significant ($P=0.0108$) on the extrinsic stiffness, control females had stiffness greater than only both thymectomized groups. Since the energy-to-fracture was similar in all groups, there were no statistically significant interactions between sex and treatment ($P=0.6283$). Treatment influenced the bone breaking strength during this period ($P<0.0001$), for both male and female control were bigger than both thymectomized-groups. In adulthood animals, the extrinsic stiffness showed a significant interaction between sex and treatment ($P=0.0003$), control females had higher results than males control and neonatally-thymectomized females. The energy-to-fracture was higher in both groups of males compared to the group of neonatally-thymectomized females, and neonatally-thymectomized males still had, higher values than the females control group ($P<0.0001$). The results showed significant interaction between treatment and sex ($P=0.0110$) for bone breaking strength, control males and neonatally-thymectomized females showed higher values than the control group females. The results show that neonatal thymectomy during the critical period of life, induce sex- and age-related modification in skeletal development and provide new insight into the dynamic complexity the skeletal sexual dimorphism.

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Bone development/growth and fracture repair

P43

Serum of patients with active rheumatoid arthritis inhibits differentiation of osteochondrogenic precursor cells

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Delayed fracture healing is frequently experienced in patients with systemic inflammation such as during rheumatoid arthritis (RA). The reasons for this are diverse, but could also be caused by inflammatory cytokines and/or growth factors in serum from patients with active disease. We hypothesized that serum from patients with active RA contains circulating inflammatory factors that inhibit differentiation of osteochondrogenic precursors.
Serum was obtained from 15 patients with active RA (active RA-sera) and from the same patients in clinical remission 1 year later (remission RA-sera; controls). The effect of active RA-sera on osteochondrogenic differentiation of chondrogenic ATDC5 cells and primary human periosteum-derived progenitor cells was determined in micromass culture. Chondrogenic and osteogenic gene expression was analysed by qPCR. We analysed cartilage matrix accumulation by alcian blue staining, and matrix mineralization by alizarin red staining. Metabolic activity of cells was analysed by using presto blue assay.
In ATDC5 cells, active RA-sera reduced Ki67 transcription levels by 40% and cartilage matrix accumulation by 14% at day 14, and Alp transcription levels by 16%, and matrix mineralization by 17% at day 21 compared to remission RA-sera. In human periosteum-derived progenitor cells, active RA-sera inhibited metabolic activity by 8%, SOX9 transcription levels by 14%, and cartilage matrix accumulation by 7% at day 7 compared to remission RA-sera.
In conclusion, our study shows that active RA-sera inhibit osteochondrogenic differentiation of precursor cells. These findings provide important new insight regarding the role of factors present in the serum of patients with systemic inflammation in delayed fracture healing, and suggest that mitigation of systemic inflammation, by agents that do not affect fracture healing directly themselves (e.g. COX2 inhibitors), might rescue delayed fracture healing in patients with systemic inflammatory disease.

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Effect of a mixture of GOS/FOS® on calcium (Ca) and phosphorus (P) absorption and bone retention in ovariectomized osteopenic rats fed a low calcium diet

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Dietary Ca insufficiency is a common finding, independently of socio-economic status. A low Ca intake (CaI) increases bone turnover leading to bone loss. This effect becomes more important during estrogen withdrawing. We previously found that a mixture of galactooligosaccharides (GOS) and fructooligosaccharides (FOS) enhances Ca and P absorption (Abs), being a suitable tool to optimize its bioavailability and consequently bone health. The present study evaluated the effect of feeding a low Ca diet containing a mixture of GOS/FOS (9:1) on Ca and P Abs and bone retention in female Wistar osteopenic rats. Rats were ovariectomized (OVX) or SHAM operated and remained untreated during a 45 day-period. Then, they were randomly assigned to received one of the following diets prepared according to the American Institute of Nutrition formulation for maintenance (AIN′93): AIN′93 (C) or AIN′93 + 2.5% GOS/FOS® (P) containing 0.5% or 0.3% of Ca (groups: C0.5%, C0.3%, P0.5% and P0.3%, respectively) for an additional period of 45-days. Dietary consumption was evaluated 3 times per week. Body weight (BW) was recorded weekly. Ca and P Abs percentage (%) were determined at the beginning and during the last 3 days of the experience. At the end of the study (tf) CTX (ng/ml): bone alkaline fosfatase (BALP) (IU/l), total skeleton bone mineral content/BW (tsBMC) (g/100 gBW) and density (tsBMD) (mg/cm^2), tibia and spine BMDs and Growth plate cartilage height (GPC.Th) (μm), were evaluated. Results at tf (mean ± SD): C0.5%; C0.3%; P0.5%; P0.3%, respectively (Different letters indicate a $P < 0.05$) BW (g): 329 ± 41; 327 ± 31; 326 ± 45; 321 ± 42; Food intake (g/d): 22.6 ± 4.9; 19.719.7.8; 22.9 ± 0.6; 20.7 ± 1.7; Ca Abs (%): 69.1 ± 3.1d; 76.2 ± 2.6c; 83.6 ± 4.6b; 89.5 ± 3.8a; P Abs (%): 73.1 ± 2.0b; 71.5 ± 1.1b; 84.8 ± 2.4a; 83.3 ± 2.1a; Caecal pH: 7.15 ± 0.10a; 7.18 ± 0.20a; 6.71 ± 0.20b; 6.67 ± 0.20b; CTX38.7 ± 5.4a; 52.2 ± 5.6b; 30.0 ± 11.0a; 41.1 ± 8.8a; BALP: 50 ± 4; 49.6 ± 6; 502417; 49 ± 6; tsBMC: 2.01 ± 0.13b; 1.78 ± 0.14c; 2.45 ± 0.10a; 2.06 ± 0.24b; tsBMD: 294 ± 7; 286 ± 6; 295 ± 2; 289 ± 2; Tibia BMD: 247 ± 10b; 231 ± 10c; 256 ± 7a; 241 ± 10b; spine BMD: 229 ± 13b; 2419 ± 12c; 241 ± 14a; 228 ± 14b; GPC.Th: 246 ± 27a; 219 ± 17b; 252 ± 20a; 245 ± 22a.
Conclusion
The consumption of the GOS/FOS® mixture added to a low Ca diet enhanced Ca and P Abs % increasing bone mass and trabecular bone density, evidencing its usefulness to give an extra amount of Ca and P for maintaining bone health. Thanks to ® N.V.Nutricia for the mixture. Grants: PIP 11220100100004 and UBACyT 20020130100091BA.

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P45

Mast cells regulate inflammation and bone regeneration in fracture healing

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Mast cells (MCs) are pro-inflammatory sensor and effector cells of the immune system. MCs seem to play a role in bone metabolism, because patients with systemic mastocytosis develop osteoporosis. MCs are present in the fracture callus during bone healing, however, their function has not yet been investigated. Here, we examined the role of MCs in the inflammatory and repair phase during fracture healing.
Male 12-week old MC deficient mice (Mcpt5-Cre R-DTA) and wildtype mice (WT) received a femur osteotomy, stabilized by an external fixator. After 1 day, the fracture hematoma was analyzed for inflammatory mediators (multiplex immunoassay) and immune cells (flow cytometry). Femur sections were subjected to histomorphometry (day 7, 14, 23). Osteoclasts were evaluated using tartrate-resistant acid phosphatase staining (TRAP). The healing outcome was assessed at day 23 by biomechanical testing (three-point bending). The study was approved by the National Ethical Committee, Germany, no. 1149). Statistics: Student's *t*-test.
Neutrophil recruitment to the fracture hematoma of MC deficient mice was diminished compared to WT (−66%, $P < 0.05$). Pro-inflammatory cytokines including IL-1β, IL-6, and TNF-α were significantly reduced (−54, −58, and −45%, $P < 0.005$). The bending stiffness of the healed bone of MC deficient mice was significantly increased (+147%, $P < 0.01$). The bone fraction was also increased (+53%, $P < 0.005$) whereas the osteoclast number was reduced in the peripheral fracture callus (N.Oc/BS, 22%, $P < 0.005$) indicating reduced bone remodeling in MC deficient mice.
In conclusion, MC deficiency reduced neutrophil recruitment and pro-inflammatory cytokines in the fracture hematoma and increased the bone content in the peripheral fracture callus probably due to reduced callus remodeling. These results demonstrate that MCs may be important regulators in the early inflammatory as well as in the repair and remodeling phase of fracture healing.

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P46

Bone regeneration using transcript-activated matrices for sustained messenger RNA delivery

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Transcript therapies, using chemically modified messenger RNAs (cmRNAs), are emerging as safer yet promising substitutes for gene and recombinant protein therapies. However, their applications have been limited due to transient translation and relative low stability of cmRNAs, compared to DNAs. Here we showed that vacuum-dried cmRNA-loaded collagen sponges, so called Transcript-Activated Matrices (TAMs), could serve as depots for sustained cmRNA delivery, providing steady state protein expression for six following days. Another advantage of this technology was high cell transfection efficacy (close to 100%) and low cell toxicity. Considering stability issues, cmRNAs on TAMs were stable at least for 6 months at RT. At the end, bone regeneration *in vitro* (with MC3T3-E1 cells and MSCs), and *in vivo* (in rat femur defects), using hBMP2 cmRNAs, confirmed the ability of TAMs in a preclinical application. In total, this study introduces TAMs as stable and efficient sustained cmRNA delivery systems for bone regeneration using hBMP2 cmRNAs.

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P47

Bone-forming ability of recombinant human bone morphogenetic protein 2 after repeated administration in mice

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Delivery of recombinant human bone morphogenetic protein (rhBMP2) with various carriers has been showing the successful induction of bone formation in many bony defects, including oral and maxillofacial regions. However, effectiveness of the exogenous proteins, when repeatedly administered into different regions in an individual, has not been determined. The present study was aimed to examine alterations of ectopic or orthotopic bone generation and serum level of anti-BMP2 antibody following the repeated administration of rhBMP2. Absorbable collagen sponge or thermosensitive hydrogel containing rhBMP2 (7 μg) was subcutaneously implanted or injected twice at 4 week intervals into both sides of the back in C57BL/6 mice, respectively. Microradiographic and histological analyses showed that the second administration of rhBMP2 (7 μg) also induced the same amount of heterotopic bone in the subcutaneous regions compared to the first administration, regardless of carrier type. When the same amount of rhBMP2 was primarily delivered with the hydrogel into subcutaneous region of the back and 3 weeks later another rhBMP2 was implanted with absorbable collagen sponge into calvarial defects, the defects were completely recovered with newly regenerated bones at three additional weeks after the second implantation. In addition, indirect ELISA assays showed that repetition of rhBMP2 did not significantly alter the blood level of anti-BMP2 antibody compared with untreated control groups in both subcutaneous and calvarial deliveries. These results suggest that rhBMP2 can be repeatedly used for bone regeneration therapy in the limits of short interval and a specific dose.

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P48

Biocompatibility of hydroxyapatite derived from whitemouth croaker (*Micropogonias furnieri*)

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The aim of this study was to investigate the biocompatibility of hydroxyapatite (HAP) powder from whitemouth croaker fish (*Micropogonias furnieri*). For this purpose, fragments from HAP with 0.5 cm^2 were inserted in the subcutaneous tissue of animals. After 7, 15, and 30 days, histopathological analysis was performed. The results showed that it was possible to detect tissue reactions closely related to cytotoxicity in a time-exposure manner. At day 7, moderate to intense inflammatory process as a result of interstitial edema, a good deal of mononuclear inflammatory cells (lymphocytes), congested vessels and the presence of biomaterial was detected. Furthermore, the histological sections were characterized for the presence of smooth collagen fibers and few fibroblasts. At day 14, a regression of the inflammation was observed, in most sections. At 30 days, few fragments of HAP surrounded by giant multinucleated cells were also observed in this period. A well-organized connective tissue was detected, with tissue proliferation into the biomaterial in some of the cases. Taken together, our results demonstrated that HAP from whitemouth croaker exhibits a great potential for using as biomaterial towards highly valuable commercial product.

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P49

The effects of combined teriparatide and denosumab on bone regeneration

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Objective
The purpose of this study is to investigate the effects of the combined teriparatide (TPTD) and denosumab (DMAB) on both cortical and cancellous bone regeneration.
Materials and methods
Bone defects were created in the diaphysis of the right femur and in the epimetaphysis of the left femur in 8-week old female C57/BL6N mice. After making bone defects, Mice were given either saline or 40 μg/kg TPTD for 20 days (5× per week) and were simultaneously given either single-shot saline or 5 mg/kg DMAB. The following assessments were performed in the four groups (Control vs TPTD vs DMAB vs COMB): time-course bone microstructural analysis of both cortical and cancellous bone defects, with *in vivo* microcomputed tomography (μCT); histological and biomechanical analysis of both cortical and cancellous bone defects after euthanasia.
Result
In the epimetaphysis, the bone defect was dramatically healed over time in the COMB group, and the bone mass of newly formed cancellous bone in the COMB group significantly increased compared to the other 3 groups 15 days post-operatively (Control: 0.01, TPTD: 0.12, DMAB: 0.17, COMB: 0.34; $P<0.05$, unit: mm^3). In the diaphysis, there were no significant differences in the bone mass of newly formed cortical bone among four groups over time. Histologically, many woven bones and cartilage matrix still existed and lamellar bone was not well formed in the both COMB and DMAB group.
Conclusion
These results suggest that combined TPTD and DMAB promotes the bone healing in the cancellous bone rich area like spine.

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P50

Prostaglandin IP agonist promotes osteoblastic differentiation and BMP induced bone formation

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Introduction
A synthetic prostacyclin IP receptor agonist (ONO-1301) has been reported to induce the production of endogenous HGF and VEGF in fibroblasts by stimulating cAMP production.
Materials and methods
In vitro; murine primary osteoblasts and cell line (ST2, MC3T3-E1, C2C12) were treated with BMP-2 (0–100 ng/ml) and ONO-1301 (0–10^{-6} M). ALP assay and cell proliferation assay were performed. In vivo analysis, Collagen pellets containing BMP-2 (1 μg/3 μg) w/ or w/o ONO-1301 were implanted into the dorsal muscle pouch of mice ($n=32$), and BV by μCT at 3 weeks after operation were evaluated. In a rat spinal fusion model, SD rats ($n=20$) were treated with 0.5 μg of BMP-2 w/ or w/o ONO-1301, and fusion rates and newly formed BV were evaluated by micro CT every week until post-op. 6w. A manual palpation test was performed.
Results
Treatment of ONO-1301 significantly increased ALP activities on primary osteoblasts and ST2. And the co-administration with BMP-2 further enhanced the effects in primary osteoblasts, ST2 and MC3T3-E1. Cell proliferation was not affected by the administration of ONO-1301. In a mouse transplantation model,

ONO-1301 administration significantly increased BV when co-transplanted with 1 μg of BMP-2 (BV [w/o ONO-1301 v.s. w/ ONO-1301; 4.3±2.8 mm^3 vs 7.0±3.9 mm^3, $P=0.05$). In a rat spinal fusion model, ONO-1301 significantly increased the BV of the newly formed bone at 4–5 weeks after operation (4w; 3.7±1.5 mm^3 vs 5.2±1.5 mm^3; $P=0.01$, 5w; 3.4±1.4 mm^3 vs 4.8±1.5 mm^3; $P<0.05$). In addition, ONO-1301 significantly increased the fusion rates (w/o ONO-1301:30% vs w/ ONO-1301:70%, $P<0.05$) and fusion analysis with a manual palpation test (w/o ONO-1301: 30%, w/ ONO-1301: 66.6% vs, $P<0.05$).
Conclusions
ONO-1301 significantly increased osteoblastic differentiation *in vitro*, and significantly enhanced ectopic and orthotopic bone formation *in vivo*. These results suggest that ONO-1301 has a potential to be used as a bone graft substitute or bone fusion enhancer in a clinical setting.

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P51

Rictor plays a critical role in bone mass and strength with the involvement of mtorc2 pathway in osteoblasts

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Mammalian target of rapamycin (mTOR) functions mainly in the form of two complexes, namely mTORC1 and mTORC2, which are distinct in their unique components, raptor and rictor. Here, we focused on bone phenotypes in mice with a specific deletion of rictor using a Cre recombinase gene whose expression was driven by the promoter of osteocalcin. All procedures involving mice were approved by the Institutional Animal Care and Use Committee of the local admin. DXA analysis showed a significant reduction in BMD of the $Rictor_{ob}^{-/-}$ mice (53.5 *vs* 59.3 mg/cm^2, $P<0.001$). Furthermore, micro-computed tomography, histomorphometric, and molecular biological analyses revealed a marked impairment of the cortical bone growth, as well as minor changes in trabecular bone, of the $Rictor_{ob}^{-/-}$ mice. Cortical tissue mass (1138.17 *vs* 1179.52 mg/ccmHA, $P<0.01$) and thickness (162.4 *vs* 193.6 m, $P<0.001$) of the femoral mid-shaft were dramatically reduced, with unusual increases in porosity (0.69 *vs* 0.36%, $P<0.01$) and marrow area (0.98 *vs* 0.91 mm^2, $P<0.05$) by micro-CT. These changes were associated with significantly decreased bone mechanical properties of the femurs as reflected by reduced peak load (15.03 *vs* 20.54 N, $P<0.001$) and stiffness (43.47 *vs* 58.98 N/mm, $P<0.001$). Thinner trabeculae were found in the lumbar spine (25.81 *vs* 33.22 m, $P<0.001$) with relatively normal structural indices of trabecular numbers and separation by histomorphometry. However, there were no significant changes in the trabecular bone of the distal femur by micro-CT. A lower rate of bone turnover was observed, as the consequence of the decreased individual osteoblast and osteoclast activities. Furthermore, osteoblast differentiation was reduced, with down-regulation of mTORC2 signaling activity as shown in primary cultures of osteoblasts that did not contain rictor. In conclusion, expression of rictor in osteoblasts is essential for the maintenance of normal bone modeling/remodeling and bone mass, especially for the normal accruement of cortical bone.

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P52

Effect of intermittent administration of teriparatide (PTH 1-34) on BMP induced bone regeneration in a rat critical-sized femoral defect model

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Introduction
Indirect inhibition of the extracellular BMP antagonist network and the activation of Wnt signaling by PTH1-34 suggest the possibility of synergistic effect on bone regeneration by the co-administration of BMP and PTH1-34.
Materials and methods
In a rat critical-sized femoral defect (7 mm) model, SD rats ($n=32$) were operated by absorbable collagen sponge containing two different BMP-2 dosage treatments; 1) 2 μg (low dose) and 2) 50 μg (high dose). Each of the BMP treatments was studied in combination with intermittent PTH1-34 (180 μg/kg per w) or saline injection starting 2 w before the operation and continuing until 8 w after operation. Microstructural indices (TV, BV, BV/TV, Tb.Th) of the newly formed bone and and fusion status were evaluated by μCT (post-op. 0, 1, 2, 4, 6, 8 w). The serum markers of bone metabolism were also quantified (post-op. 4 and 8 w).
Results
Fusion rates at post-op. 8w were not significantly different w/ or w/o PTH1-34 administration. (Saline/PTH1-34: BMP; 2 μg 75%/100%,50 μg; 100%/100%). Microstructural indices of the newly formed bone (BV/TV, Tb.Th) were signifi2cantly improved by PTH1-34 administration in both BMP dose groups. Time-dependent changes demonstrated that the TV in the BMP high dose group significantly increased by PTH1-34 administration (PTH1-34: 77.6±29.2 mm^3, saline: 34.6±4.9 mm^3, $P<0.001$). Serum levels of osteocalcin and P1NP were significantly increased by PTH1-34 in both BMP dose groups (BMP low group: $P<0.01$, BMP high group $P<0.05$).
Discussion and conclusions
Intermittent administration of PTH1-34 significantly increased the quality of the newly formed bone in the BMP treatment groups. However, the remodeling of newly formed bone in BMP high dose group was not apparent in this study (with internal fixation) compared to that in our previous rat spinal fusion model (without fixation) (Morimoto *et al.* 2014). PTH may accelerate the remodeling process of the newly formed bone in response to mechanical stress.

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P53

Effect of bioactive glass-ceramic scaffold associated with bone marrow - or adipose-derived mesenchymal stem cells on bone formation under osteoporotic conditions

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In this study, we evaluated the effect of the association of a bioactive glass-ceramic scaffold (Biosca) with mesenchymal stem cells derived from either bone marrow (BM-MSCs) or adipose tissue (AT-MSCs) on bone formation in calvarial defects of osteoporotic rats. Wistar rats were submitted to bilateral ovariectomy (OVX) or only to the surgical stress (Sham), under approval of the Committee of Ethics in Animal Research. After 5 months, 5-mm unilateral calvarial defect was created and filled with Biosca combined with BM-MSCs or AT-MSCs. Biosca without cells and empty defects were used as controls. After 4 weeks, the calvariae were harvested, fixed and analysed by microtomography to evaluate bone volume (BV – mm^3), percentage of bone volume (%BV) and bone surface (BS – mm^2). Data were compared using ANOVA test ($P\leq0.05$). For OVX and Sham rats, BV was, respectively, 1.91±0.64 and 1.30±0.70 in Biosca with BM-MSCs, 2.13±0.77 and 1.50±0.75 in Biosca with AT-MSCs, 2.88±1.14 and 2.49±1.12 in Biosca, and 0.40±0.55 and 0.47±0.03 in empty defects. The %BV was, respectively, 6.45±2.18 and 4.40±2.36 in Biosca with BM-MSCs, 7.22±2.62 and 5.07±2.53 in Biosca with AT-MSCs, 9.57±3.87 and 8.40±3.8 in Biosca, and 1.37±1.85 and 1.61±0.12 in empty defects. The BS was, respectively, 297.29±84.13 and 210.59±116.29 in Biosca with BM-MSCs, 310.75±105.46 and 240.58±126.63 in Biosca with AT-MSCs, 377.97±131.00 and 413.21±177.84 in Biosca, and 40.22±54.06 and 33.69±2.03 in empty defects. No significant differences were observed among the treated defects related to the use of cells, cell source or OVX and Sham for all evaluated parameters. In conclusion, all treatments induced meaningful higher bone formation compared with empty defects, irrespective of OVX and Sham. We observed that Biosca is capable of increasing bone formation either in OVX or Sham rats, that is not enhanced by the association with MSCs.

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P54

Role of interleukin-6 in the early and late fracture healing phase

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Interleukin-6 (IL-6) plays an important role in bone metabolism and regulates fracture healing in a presently unknown process. In the fracture callus IL-6 expression is biphasic; it peaks during the inflammatory phase and again during intramembranous and endochondral ossification (Ai-Aql *et al.* 2008). Few studies using IL-6 knockout mice indicate that IL-6 might be crucial for bone healing (Yang *et al.* 2007). However, a generalized IL-6 knockout induces multiple organ dysfunctions hampering the specific analysis of IL-6 action in bone healing. Here, we investigated the role of IL-6 signaling in the inflammatory and bone repair phase using a pharmacological approach.

Male C57BL/6J mice received a femur osteotomy. The animals were injected every second day with either IL-6 (IL-6 Ab) or IgG antibody (IgG Ab) during the early postoperative phase (day 1–3) or during the repair phase (day 7–17). After 21 days fracture healing was assessed by biomechanical testing, μCT and histomorphometry ($n=3$–9; $P=0.05$; Student's *t*-test).

Blockade of IL-6 signaling in early phase significantly decreased the biomechanical properties of the fracture callus. μCT analysis and histomorphometry revealed a reduced bone fraction after IL-6 Ab treatment. In contrast, blockade of the IL-6 in the later healing phase significantly increased callus size and relative bone fraction. Additionally, flexural rigidity was increased by trend. Concluding, blockade of IL-6 signaling in inflammatory phase impaired fracture healing. Because IL-6 mediates both pro- and anti-inflammatory effects (Waetzig *et al.* 2012), it can be suggested that IL-6 might be crucial for inducing downstream responses leading to fracture repair. In contrast, the inhibition of IL6 in the later healing phase increased callus size and bone fraction presumably by reducing bone remodelling, as IL-6 is known to indirectly promote osteoclastogenesis (Blanchard *et al.* 2009). Further molecular and cellular analyses are on going to elucidate the underlying mechanisms.

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P55

Calcium and vitamin-D supplementation post-trauma improves bone healing and decreases posttraumatic bone resorption in an osteoporotic mouse model

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Chronic calcium- and vitamin-D-deficiencies are crucial risk factors for osteoporosis. However, their significance for fracture healing is still poorly investigated, despite the clinical evidence that osteoporotic bone healing is disturbed. This study addressed the important question, whether chronic deprivation of calcium and vitamin D compromises bone repair and if this could be rescued by a supplementation post-trauma. Because clinical hints suggest that a fracture induces general bone loss in the skeleton, thus further increasing fracture risk, we also analyzed, if a fracture induces bone loss particularly under deficiency conditions and if this can be prevented by a specific supplementation therapy.

Female C57BL/6J mice were ovarectomized to induce an osteoporotic phenotype. One group was fed a standard-diet and second group a calcium/vitamin-D-deficient-diet. After 8 weeks, all mice received a femur osteotomy. Half of the group with chronic calcium/vitamin-D-deficiency was supplemented with calcium/vitamin-D post-trauma. Bone healing and the general bone status were assessed at day 10 and 23 by biomechanical analysis, μCT, histomorphometry and serum analysis. To evaluate posttraumatic bone turnover, intact skeleton of fractured mice was compared to non-fractured mice. $n=4$–10; $P<0.05$; ANOVA/LSD.

Calcium/vitamin-D-deficiency impaired fracture healing as confirmed by significantly decreased bone content in the fracture callus (−18%). Furthermore, fractured mice of the deficient-group exhibited significantly more osteoclasts in the callus and intact skeleton compared to standard-group or non-fractured mice, respectively, indicating posttraumatic bone loss. Calcium/vitamin-D-supplementation abolished the negative effects as demonstrated by significantly improved bone formation and reduced osteoclast activity. In the serum, FGF-23 was increased and CTX was decreased ($P<0.05$).

Calcium/vitamin-D-deficiency disturbed osteoporotic fracture healing accompanied by increased osteoclast activity. Supplementation abolished these effects possibly mediated through FGF-23 that was already shown to inhibit osteoclast formation. The results support the therapeutic potential for calcium/vitamin-D-supplementation to enhance osteoporotic fracture healing and prevent posttraumatic bone loss in the clinics.

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P56

Morphological and densitometric properties of tarsometatarsus in adult male and female emu (*Dromaius Novaehollandiae*)

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Considering limited information on skeletal system properties in emu (Dromaius novaehollandiae), the aim of this study was to determine morphological and densitometric parameters of tarsometatarsus obtained from adult males ($N=6$) and females ($N=7$). The study was performed on birds reared at the Department of Poultry and Ornamental Bird Breeding of Western Pomeranian University of Technology in Szczecin, Poland. Male and female emu were slaughtered to obtain left tarsometatarsus for analyses. Bone weight, relative bone weight and bone length were determined. Using quantitative computed tomography (QCT) technique and self-determined regions of interest
(ROIs), volumetric bone mineral density (vBMD) of trabecular (Td) and cortical bone (Cd), as well as cortical bone area (CBA) were measured. Td was determined in proximal epiphysis, while Cd was measured in the midshaft. Automatically defined ROIs were used to determine mean Td, mean Cd and mean CBA. Moreover, total bone volume (Bvol) and mean volumetric bone mineral density (MvBMD) were determined. All QCT measurements were performed using LightSpeed VCT scanner (GE Medical Systems, USA) and OsiriX software for Mac Pro 29-ZRL computer. Statistical analysis was performed using paired Student *t*-test and $P<0.05$ was statistically significant. Final body weight was significantly higher by 25% in females than in males ($P=0.01$). Significantly higher values of bone weight, bone length, Bvol, and mean CBA of tarsometatarsus were found in females, when compared to males ($P<0.01$).

In conclusion, this study has shown sex-related differences of body weight and morphological properties of tarsometatarsus in emu, while the investigated densitometric parameters were not sex-differentiated. This study provided data on morphological and densitometric properties of tarsometatarsus in emu. The obtained results indicate that emu may serve as an experimental model for further studies on bone metabolism regulation in vertebrates with the use of physiological, environmental, pharmacological, nutritional and toxicological factors influencing skeletal system properties.

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P57

Interrelationships between morphometric, densitometric and mechanical properties of femur in ostriches (*Struthio Camelus*)

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The aim of the study was to evaluate interrelationships between morphometric, densitometric and mechanical properties of femur in ostriches (Struthio camelus). 14-month-old male and female ostriches ($N=46$) were slaughtered to obtain left femur for analyses. Using quantitative computed tomography (QCT) technique, volumetric bone mineral density (vBMD) of the trabecular (Td) and cortical bone (Cd) were measured. Bone mineral density (BMD) and bone mineral content (BMC) were determined using dual-energy X-ray absorptiometry (DEXA) method. 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 femur in the midshaft. Using three-point bending test, mechanical parameters such as maximum elastic strength (Wy) and ultimate strength (Wf) of femur were determined. Pearson's correlation coefficient (r) was determined between all the investigated variables and $P<0.05$ was considered as statistically significant. Positive correlations of bone weight, bone length, BMC and Ix with final body weight were found ($P<0.05$). Bone weight was positively correlated with bone length, BMD, BMC, A, Ix, MRWT and CI ($P<0.05$). Td was positively correlated with Cd, BMD and Wy ($P<0.05$). BMD was positively correlated with BMC, A, Ix, MRWT, CI, Wy and Wf ($P<0.05$). BMC was positively corretated with bone length, A, Ix and Wy ($P<0.05$). Cross-sectional area was positvely correlated with all the investigated geometrical and mechanical parameters, while Ix was positively correlated with CI, Wy and Wf ($P<0.05$). Positive correlations between MRWT and CI, as well as between Wy and Wf were found ($P<0.05$).

In conclusion, this study showed numerous interrelationships between morphometric, densitometric and mechanical properties of femur in ostrich. These results indicate that femur from ostrich may be used as an experimental model for further studies on metabolic response of skeletal system to environmental, physiological, nutritional, toxicological and pharmacological factors.

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P58

Loss of androgen receptor suppresses chondrogenic proliferation during endochondral ossification in mice

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There have been considerable advances in our knowledge of how loss of androgens signalling leads to bone loss in aging male with hypogonadism. However, the roles of androgen actions on the skeletal growth remain limited. Recently, new insights into the function of and androgen receptor (AR), learned from human genetic mutations and gene targeting mouse models have contributed to emerged understanding of the androgenic effects on bone and cartilage in health and diseases. Here, we studied the roles of the androgen/AR signalling in regulating stem/progenitor cells by which the androgenic steroids act as anabolic hormones on promotion of bone growth. Lineage-tracing experiments revealed that osteochondroprogenitors expressing type II collage (*Col2*) gene encompasses early mesenchymal progenitor cells, which preferentially become chondrocytes. Inactivation of AR by *cre* recombinases driven by the *Col2a1* promoter causes delayed endochondral bone formation, impaired chondrocyte proliferation and leading to a smaller skeleton in mice. Col2-ARKO male mice have shorter bone length compared to wild type mice. Mechanistically, AR promotes chondrogenic *IGF-1* gene expression by demethylating H3-K27, thereby leading to promote proliferation of chondrogenic cells. AR silencing decreased proliferation of chondrogenic cells could be rescued by activation of IGF-1 signal. Together, these results establish AR as a key regulator of chondrogenesis and provide insights into the therapeutic potential of targeting AR in treating bone and cartilage-associated diseases.

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P59

Midkine is involved in the pathogenesis of delayed osteoporotic fracture healing after ovariectomy in mice

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Previous studies demonstrated a negative influence of the growth- and differentiation factor midkine (MDK) on bone formation during bone remodeling and fracture healing. Additionally, MDK-deficiency protected mice from a loss of trabecular bone mass after ovariectomy (OVX). Therefore, we hypothesized that MDK may also be involved in the pathogenesis of delayed, osteoporotic fracture healing after OVX in mice. Thus, we analyzed the expression of MDK during bone regeneration and the effects of MDK-antibody (MDK-Ab) therapy on both bone healing and the intact skeleton in OVX-mice.

12-weeks-old, female C57BL/6J mice were either sham-operated or ovariectomized. 8 weeks later, all animals received a femur osteotomy and were treated with vehicle or MDK-Ab twice per week for three weeks. The mice were sacrificed at day 3, 10 or 23 and the fractured and intact femurs were assessed by 3-point-bending-test, μCT or immunohistochemical analysis. MDK serum levels were determined by ELISA.

Interestingly, OVX-mice displayed significantly increased MDK serum levels at day 10 (62 pg/ml) in comparison to control-mice (<5 pg/ml) and showed significantly delayed fracture healing. Treatment with MDK-Ab abolished the increase in MDK serum level and led to a significantly increased relative flexural rigidity (+92%) and bone volume fraction (+38%) in the callus of OVX-mice at day 23. Immunohistochemical staining demonstrated a significantly higher expression of beta-catenin in the fracture callus of treated OVX-mice (+122%). Additionally, MDK-Ab treatment led to a significantly increased trabecular bone volume (+58%) and trabecular thickness (+10%) in the intact femurs of OVX-mice.

We conclude that circulating MDK is involved in the pathophysiology of delayed fracture healing after OVX. Antagonizing MDK increased the beta-catenin signaling, leading to a faster callus mineralization and therefore to an accelerated bone repair. This indicates a therapeutic potential for the MDK-Ab to enhance fracture healing in patients with delayed regeneration due to postmenopausal osteoporosis.

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P60

Role of PHOSPHO1 in chondrocyte matrix vesicle mineralization: an AFM study

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We used atomic force microscopy (AFM) to study the morphology and development of mineralization-competent matrix vesicles (MVs) secreted by chondrocytes isolated from WT and *Phospho1*$^{-/-}$ mice in order to validate the role of PHOSPHO1 in MV mineralization. All MVs appeared as flattened globular features either individually dispersed or connected to a mat-like structure. The mat-like structure very closely resembled type-X collagen that has been described by others. WT MVs were in greater number than *Phospho1*$^{-/-}$ MVs and showed volumes ranging from few to hundreds of thousands of cubic nanometers. *Phospho1*$^{-/-}$ MVs showed volumes in a smaller range of values. AFM topographic and phase analyses showed that WT MVs with volumes smaller than $\sim 5\times10^3$ nm^3 had a smooth surface and phase angle (φ) values that were almost constant and slightly smaller than those of mica substrate. On the contrary, bigger WT MVs showed a non-uniform surface with several angstrom tall irregularities and a great heterogeneity of φ values with spots that had φ values similar to those of mica substrate and were surrounded by regions with negative φ values. These spots corresponded to height irregularities in topographic images and their φ values increased with MV volume. We interpreted these spots as caused by the presence of the nucleational core (NC) under the MVs' membrane. *Phospho1*$^{-/-}$ MV surface was smooth for all vesicles and showed small irregularities only for bigger vesicles. *Phospho1*$^{-/-}$ MV φ values were constant, slightly bigger than those of mica substrate and increasing with MV volume, thus suggesting the absence of a NC in most of these MVs. Raman spectroscopy validated the differences between the material inside WT and *Phospho1*$^{-/-}$ MVs. Taken together our data document the decreased ability of *Phospho1*$^{-/-}$ MVs to initiate mineralization.

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P61

Advanced phase gestational jet lag reduces bone mass of adult offspring

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The mammalian circadian clock is tightly controlled by clock genes, which have been shown to regulate up to 20% of the transcriptome in any given tissue. Evidence is accumulating that light-modulation perpetually affects circadian clock performance. In accordance, shift work or chronic jet lag is associated with higher risk of disease later in life, including osteoporosis. In this study, we assessed whether gestational jet lag in mice reduces bone mass postnatally.
During gestation, pregnant mice were randomly subjected to either a constant (CON) 12 h light:12 h dark (LD) cycle or to 8-h advance phases (ADV) or delayed phases (DEL) of the LD cycle every 3 days. Male offspring was on a CON LD cycle from birth until 24 weeks of age. Femoral bone mass was assessed by microcomputed tomography and bone strength by three-point bending.
Offspring from pregnant mice on an ADV regimen showed reduced trabecular and cortical bone mass compared to those undergoing a CON regimen. Especially in the diaphysis, endocortical volume (0.37 ± 0.002 vs 0.45 ± 0.01 mm^3; $P = 0.004$), perimeter (4.88 ± 0.03 vs 5.19 ± 0.05 mm; $P = 0.02$) and moment of inertia (0.28 ± 0.001 vs 0.34 ± 0.01 mm^4; $P = 0.014$), a proxy for bone strength, were significantly reduced in ADV offspring, whereas cortical thickness was elevated (152.6 ± 1.7 vs 146.5 ± 1.8 mm; $P = 0.05$). This was corroborated by reduced stiffness and work to failure in the ADV offspring, although not reaching significance. In contrast to the ADV mice, none of the parameters for the DEL offspring was different from the CON offspring.
We have provided evidence that advanced gestational jet lag reduces bone mass of male adult offspring, which may reflect reduced bone turnover but could also implicate delayed aging. Alternatively, these mice may have compromised periosteal apposition. The skeletal consequences at the cellular level as well as the epigenetic landscaping caused by the different LD cycles remain to be scrutinised.

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P62

Bcl-2-associated athanogene-1 (BAG-1) regulates chondrocyte and osteoblast development

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The co-chaperone, Bcl-2-associated athanogene-1 (BAG-1), is expressed by chondrocytes and osteogenic cells, and interacts via heat shock chaperones (HSC70/HSP70) with diverse proteins such as nuclear hormone receptors to regulate cell proliferation, differentiation and apoptosis. Early embryonic lethality in *Bag-1* null mice has limited the investigation of the function of BAG-1 in skeletal development. The present study aimed to elucidate the role of BAG-1 in skeletal development by using *Bag-1* null and heterozygous mice.
Micromass cultures of limb bud mesenchymal cells of E11.5 *Bag-1*$^{-/-}$ mice demonstrated significantly high number of mineralised cartilage nodules compared to micromass cultures of limb bud mesenchymal cells of *Bag-1*$^{+/-}$ and wild-type mice. Significantly increased expression of hypertrophic genes, in combination with robust Alkaline phosphatase and Alizarin red staining of the cartilage nodules, indicated marked upregulation of chondrocyte hypertrophy and matrix mineralisation in the cartilage nodules generated by limb bud mesenchymal cells of *Bag-1*$^{-/-}$ mice. Deletion of one functional Bag-1 allele significantly decreased the osteogenic differentiation potential of bone marrow stromal cells (BMSCs) of *Bag-1*$^{+/-}$ female mice in response to BMP-2. Addition of 17-β-estradiol (E2) enhanced responsiveness of BMSCs of *Bag-1*$^{+/-}$ mice to BMP-2 and promoted robust BMP-2-stimulated osteogenic differentiation of BMSCs. BAG-1 can modulate cellular responses to E2 by assisting the establishment of functional estrogen receptors (ERs), crucially, via its interaction with HSC70/HSP70. The interaction between BAG-1 and HSC70 in BMSCs was inhibited by the small-molecule chemical inhibitor, Thioflavin-S, and the C-terminal BAG domain-derived short peptide, and resulted in significant downregulation of E2/ER-facilitated BMP-2-directed osteogenic differentiation of BMSCs.
Thus, by regulating terminal differentiation of chondrocytes and hypertrophic cartilage mineralisation, BAG-1 plays an important role in the transition from chondrogenesis to osteogenesis during endochondral ossification. The study has also demonstrated the significance of BAG-1-mediated protein-protein interactions, specifically, BAG-1-regulated activation of ER by HSC70, in the regulation of osteoblast development.

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P63

Comparison of bone stiffness during fracture healing in the human distal radius assessed with HR-pQCT using μFEA and FEA based on downscaled images

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High resolution peripheral quantitative computed tomography (HR-pQCT) in combination with micro finite element analysis (μFEA) is a promising tool to assess longitudinal changes in bone mechanical properties during the fracture healing process in the distal radius. In the present study we investigate if these changes can be detected as well when using images with lower resolutions, comparable to clinical QCT images.
Postmenopausal women with a stable distal radius fracture ($n = 17$) were scanned by a HR-pQCT system at four visits during a 12 week follow-up period. HR-pQCT scans (isotropic voxel size 82 μm) were downscaled, mimicking QCT resolutions. Downscaled voxel sizes were $164 \times 164 \times 164$, $328 \times 328 \times 328$, $656 \times 656 \times 656$, $1312 \times 1312 \times 1312$ and $328 \times 328 \times 656$ μm, respectively. Stiffness in compression, torsion and bending were assessed by μFEA based on HR-pQCT scans and by FEA based on downscaled scans, both using grey-level dependent material properties. μFEA outcomes and FEA outcomes based on the downscaled images were compared by assessment of Spearman's correlation coefficient and Bland-Altman plots. A linear mixed effect model was used to identify significant changes in stiffness from baseline. When similar significant longitudinal changes were found for μFEA and downscaled FEA, the resolution was considered sufficient to assess bone stiffness in the fracture healing process. All correlations were significant ($P < 0.05$), however decreased when a larger downscaling factor was applied. FEA outcomes with voxel sizes of $328 \times 328 \times 656$ μm and smaller deviated approximately 13, 7 and 9% from μFEA outcomes for stiffness in compression, torsion and bending, respectively. μFEA outcomes all showed significant changes from baseline at 12 weeks post-fracture ($P < 0.05$). The largest downscaled voxel size with significant longitudinal changes in stiffness was $328 \times 328 \times 656$ μm ($P < 0.1$).
Concluding, FEA based on scans with clinically feasible voxel sizes could lead to similar conclusions for torsional and bending stiffness as μFEA based on HR-pQCT images during the fracture healing process.

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P64

A novel model for examining chondral bone regenerative potential in adult zebrafish

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Purpose
The aim of this research was to develop an *in vivo* platform for conveniently examining the potential of various factors to augment chondral bone regeneration. For this purpose, we have established a de novo partial tail amputation model in adult zebrafish, which includes the resection of cartilage-template based bones from the endoskeletal caudal complex. Endoskeletal amputations were rarely studied, as opposed to those of the distal caudal fin rays, a well-established model of membranous bone regeneration.
Methods
Amputations initiated proximally at the distal 2–3 mm of the dorsal part of the tail that has scales and musculature, and then proceeded distally towards the dorsal fin rays. Fish skeletons were vitally labelled with two Ca^{2+} -binding chromophores: calcein 2 days before amputation, and 20 days later, alizarin red. To overcome the potential artifacts of autofluorescence, and the spectral overlap between both chromophores, we employed spectral imaging coupled with image analysis using the linear unmixing algorithm.
Results
After the amputations fish were vital, and normal activities were not compromised. The labelling method, which was validated in the distal caudal fin model, exhibited newly formed bone, mainly some broadening at the tip of the stump in neural spines, while hypurals did not regenerate. None of the caudal complex bones regrew to form a structure similar to the original one, even 7 months post amputation.
Conclusions
We suggest that neural spines undergo a progressive widening at the tip of the stump after amputation, which resembles hypertrophic nonunion fracture in humans, while hypurals do not seem to regenerate. Thus, our model is appropriate for the investigation of potential therapeutic factors for augmenting chondral bone regeneration, either for the induction of de-novo regeneration or for examination of treatments for abnormal regrowth; both of major importance for clinical conditions and aspects of regenerative medicine.

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P65

Effect of postponing puberty and cross-sex hormone therapy on bone turnover markers and BMAD in transgender adolescents

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Background
Puberty is highly important for the accumulation of bone mass. Bone turnover and bone mineral density can be affected in transgender adolescents when puberty is postponed by gonadotropin-releasing hormone analogues (GnRHa), followed by treatment with cross-sex hormone therapy (CSHT).
Objective
To investigate the effect of GnRHa and CSHT on bone turnover markers (BTMs) and bone mineral apparent density (BMAD) in transgender adolescents.
Methods
Thirty four female-to-males (FtMs) and 22 male-to-females (MtFs) were divided into a young and old pubertal group, based on the bone age of 14 years in the FtMs and 15 years in the MtFs. All patients received GnRHa triptorelin. CSHT was prescribed in incremental doses from the age of 16 years. FtMs received testosterone ester mixture and MtFs were treated with 17-β estradiol. BTMs P1NP, osteocalcin and ICTP and the BMD of lumbar spine (LS) and femoral neck (FN) were measured at three time points. Furthermore, BMAD and *Z*-scores were calculated.
Results
P1NP and 1CTP decreased during GnRHa treatment, indicating decreased bone turnover. Osteocalcin showed an aberrant pattern. A low BMAD *Z*-score of both FN and LS was observed in the MtFs at start of GnRHa treatment. The decrease in bone turnover upon GnRHa treatment was accompanied by a decrease of BMAD *Z*-scores of predominantly the LS. During 24 months of CSHT BTMs P1NP and ICTP decreased even more. During CSHT BMAD and *Z*-scores increased and returned towards normal, especially of the LS.
Conclusion
Postponing puberty by GnRHa leads to a decrease of BTMs in transgender adolescents. The increase of BMAD and BMAD *Z*-scores predominantly in the LS as a result of treatment with CSHT is accompanied by decreasing BTM concentrations after 24 months of CSHT. Therefore, the added value of evaluating BTMs seems to be limited and DEXA-scans remain important in follow-up of transgender adolescents.

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P66

OSTEOGROW: BMP6 device for enhance bone healing

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High doses of BMPs are needed to achieve clinical success in bone fracture repair with currently available devices. BMP6 has a higher potency in stimulation of bone formation then its paralogue BMP7 due to less susceptibility to Noggin. The BMP6 carrier is a whole blood derived coagulum (WBCD) from the peripheral blood (OSTEOGROW). This WBCD, as an endogenous biocompatible material, significantly reduces the inflammatory response associated with current BMP-based treatments. More than 90% of BMP6 added to the full blood remains incorporated, bound mainly to its extracellular matrix components. Release of BMP6 from the coagulum in *in vitro* conditions showed slow discharge from the coagulum with a mean residence time of ~7 days. Proof of concept studies have been conducted in rabbit and rat models of bone defects. These showed that BMP6 accelerated and enhanced radiographic bone union across the defect. Pharmacokinetic studies, following intravenously (iv) dosing, have been conducted in a *Bmp6* knock-out mice, rats and in rabbits. Presence of BMP6 in circulation is minimal after systemic application and BMP6 is not distributed into the deep tissue compartment. Implant studies conducted in the rat have shown negligible absolute bioavailability of paraosseally administered BMP6 in WBCD. A single dose GLP toxicology study conducted in rats showed absence of BMP6 related adverse effects when rhBMP6 was administered iv in doses up to 450 μg/kg, which is around 300-fold higher than the maximum anticipated human dose, assuming 5% bioavailability. The clinical grade of BMP6 is currently tested 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 is expected to promote faster bone healing and reduce the need for secondary interventions.

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P67

Comparison of osteoconductivity and absorbability of beta-tricalcium phosphate and hydroxyapatite in open wedge high tibial osteotomy

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The purposes of this present study were to compare the osteoconductivity and absorbability in patients of open wedge high tibial osteotomy with medially gaps filled with hydroxyapatite (HA) or beta-tricalcium phosphate (β-TCP).
Total 41 knees of 40 patients in whose follow up period more than 1 year were enrolled. The patients were divides into two groups, group I (22 knees, 21 patients) used HA and group II (19knees, 19 patients) used β-TCP.
According to proven method, the osteoconductivity was assessed by the radiographic change of osteotomy line and absorbability was evaluated using the occupied area of substitutes at immediate postoperative, postoperative 6 months and 1 year.
In each group, no statistically significant changes of lower limb alignment (mFTA, WBL%) and posterior slope between postoperative and final follow up radiographs were confirmed. Concerning the osteoconductivity, there were no significant differences between two groups in any zone. However, the absorption rate was significantly greater in the group II than in group I at 6months (group I: 13.7 ± 6.8, group II: 35.3 ± 15.8, $P = 0.001$) and 1 year (group I: 24.2 ± 6.3, group II: 49.6 ± 14.3, $P < 0.0001$). The complications related to bone substitutes were not observed.
Both HA granule and β-TCP wedge showed satisfactory gap healing without complications and can be successfully used as alternative healing materials in opening wedge high tibia osteotomy.

Keywords: osteoconductivity, absorbability, hydroxyapatite, tricalcium, phosphate open wedge high tibial osteotomy
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P68

Adiponectin prevents orthodontic tooth movement in rats

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Adiponectin may play a role in both bone and periodontal remodeling. In this study, the effect of repetitive local administration of human recombinant adiponectin on experimental tooth movement was examined.
The first molar of 24 male Wistar rats was moved mesially by a closed coil spring ligated to the molar and anchored to the incisors in front for 14 days, with adiponectin injections every 3rd day. Amount of tooth movement was examined by feeler gauge and *in vivo* μCT. Effect of adiponectin injections on bone and periodontal ligaments were examined by histology sections of the right maxilla. Influence on circulating bone markers and cytokines were studied by Luminex and ELISA.
Adiponectin injections caused a significant reduction in tooth movement on day 12 and 14 compared to control. No difference in bone mineralization was identified with μCT. Quantitation of bone volume fraction, demonstrated a reduction in the control group at day 14 whereas the bone volume in the adiponectin was unchanged, the difference, however, was not significant. Adiponectin enhanced the collagen content in the periodontal region compared to control. No significant changes in selected bone and cytokine factors in plasma were observed between in adiponectin-treated compared to control rats.
Adiponectin prevents orthodontic tooth movement in rats. This anchorage effect may be taken advantage of in clinical settings, though further studies are required.

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The effects of bone wound healing by implanting novel type I collagen scaffolds

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Background
Three-dimensional scaffolds for the tissue regeneration are also crucial materials for bone formation. In particular, the scaffolds must provide the space for the cell migration and new bone formation. Collagen is one of the useful scaffolds to reach these performance, however, it is not always a desired biodegradability. Recently, we succeeded in developing low adhesive scaffold type I collagen (LASCol) (patent pending) which has the ability to form fibrils. In this study, we report whether the implant of LASCol was effective to induce new bone formation in a critical-sized defect (CSD) of rat shinbone.
Methods
LASCol or atelocollagen was lyophilized. The cylindrical bone of 3 mm diameter was removed from right and left shinbones of SD rat (15 weeks). Subsequently, the lyophilized graft of LASCol or atelocollagen (each 3 mg) was implanted into the defect position of both shinbones. After 15 days, one of shinbones including the defected position was evaluated by histological observation with HE staining and by immunohistological definition with anti-collagen antibody. To evaluate new bone formation, micro-computed tomography (μCT) scanning of the other shinbone was carried out. In addition, we measured Gla-osteocalcin in the rat serum by ELISA kit.
Results
After 15 days of the implant operation, LASCol graft in rat was disappeared, and instead spongy bones were found in the same space. On the other hand, atelocollagen graft clearly remained in shinbone. The μCT analysis was identical to histological results. Furthermore, Gla-osteocalcin in the serum of LASCol implantation group was shown to increase. In conclusion, we showed that LASCol graft has the potential to induce osteogenic regeneration in the CSD of rat shinbone.
Funding
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P70

Is heparin effective for the controlled delivery of high dose bone morphogeneic protein-2?

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Sustained release of bone morphogeneic protein (BMP)-2 by heparin-contained biomaterials is advantageous for bone tissue regeneration using low dose BMP-2. However, its effect in high dose BMP-2 is still unclear and should be clarified considering the clinical use of high dose of BMP-2 in spine and oral surgery. This study aimed to evaluate the efficacy of heparin-conjugated collagen sponge (HCS) in high dose BMP-2 delivery by investigating in vivo initial osteogenic regulation and bone healing over time passage to 12 weeks in comparison with that of an absorbable collagen sponge (ACS). The *in vitro* BMP-2 release profile in the HCS exhibited a lower burst followed by a sustained release of BMP-2, while that of the ACS showed an initial burst phase only. As a result of lower burst, the HCS-BMP group showed more expression of bone-forming/resorbing markers and more activation of osteoclasts than the ACS-BMP group within the scaffold of defect at 7 day, which is presumed to a retention of relatively higher amount of BMP-2. However, the surrounding calvaria were less resorbed in HCS-BMP group, compared to the aggressive resorptive response in the ACS-BMP group. Micro-computed tomography and histology revealed that HCS-BMP guided more effective bone regeneration of central defect during time passage by inducing minor ossification at defect exterior while ACS-BMP exhibited excessive ossification at the defect exterior. These results showed that HCS-mediated BMP-2 delivery at high dose has advantages over ACS including less early resorption of surrounding bone tissue and higher efficacy in compact bone regeneration over a longer period, highlighting a clinical feasibility.

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P71

Transcriptional analysis of bone healing in mouse: an insight into biological and chronological overlapping genes

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The healing of skeletal fractures involves a cascade of overlapping cellular events. This study aims to deepen the understanding of molecular networks orchestrating these events.
Standard closed fracture in the left femur of male 8–10 weeks old C57BL/6N mice were analyzed at (day = D) D3, D7, D10, D14, D21 & D28 post fracture ($N = 5$/time point). Total RNA was prepared for whole genome expression profiling using Illumina μ-array kit. Data normalization was performed using the "R" platform. The thresholds for filtering the differentially expressed (DE) genes

were set at Fold-change $\geq |1|$ & P-value ≤ 0.01. Functional enrichment analysis was performed using NCBI-DAVID and Cytoscape to identify genes of immune response, mitochondria, ribosome, angiogenesis, ossification and extracellular-matrix (ECM).

This study addresses the complexity of overlapping genes involved in the above mentioned biological processes during the different stages of healing. After gene ontology, 35 genes which were DE in more than one biological processes were further analyzed. As an overlapping gene between immune response, mitochondrion and angiogenesis: Glutathione Peroxidase-1 was DE at the early and later stages of healing to support the reactive oxygen species homeostasis. Similarly, the correlated gene between ossification and ribosome: Ubiquitin-B was DE during the early and later stages to control the osteoblast differentiation. However, the angiogenesis, ECM and ossification correlated gene: Matrix metallopeptidase-2 was DE until the endochondral ossification healing phase which plays a role in the remodeling of vasculature and encodes binding of collagens.

Currently, the detailed regulatory role of these genes and their involvement in different skeletal disorders are being investigated. However, the results indicate promising understanding of overlapping gene function between the cellular events.

Bone diseases or fractures leading to delayed or nun-union healing are often treated to enhance bone formation. Therefore, investigating gene expression overlapping -both chronologically and biologically- is crucial for the design of systemic or local therapeutic agents.

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P72

Sulfated hyaluronan improves bone defect healing in type 2 diabetic rats by increasing osteoblast function

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Bone fractures of patients suffering from type 2 diabetes mellitus (T2DM) represent an emerging socioeconomic problem. Underling mechanisms are poorly understood and therapies are limited. Our previous studies have shown that sulfated hyaluronan (sHA3) suppresses osteoclast activity while supporting osteoblast function in vitro. Hence, we now investigated if sHA3 can improve the delayed fracture healing in rats with T2DM.

Porous, cross-linked lactide-based (TriLA) scaffolds were coated with collagen-based matrices including sHA3 and inserted into a subcritical femoral gap defect in non-diabetic (+/+) and diabetic (fa/fa) Zucker Diabetic Fatty (ZDF) rats. After 12 weeks, bone regeneration was assessed using μCT and histology.

As expected, diabetic ZDF rats displayed a delayed bone defect healing compared to non-diabetic controls. After 12 weeks, TriLA scaffolds showed no systemic effects except for an elevated P1NP serum concentration (+31%) in diabetic rats. The bone defect filling in T2DM increased to the level of non-diabetic control rats (with pure collagen-coating) after collagen/sHA3-coating. On the histological level, the mineralization amount increased with sHA3 (+/+: +336%, fa/fa: +151%) whereas the amount of osteoid decreased in both genotypes (+/+: −86%, fa/fa: −75%). Osteoblast-like UMR-106 and osteoclast-like RAW-264.7 cells were incubated with sHA3 and increasing glucose concentrations (40 and 100 mM). After sHA3, osteoblasts had less cell death events, while high glucose had no effect. The gene expression level of BMP2 increased (up to +182%), while the RANKL/OPG ratio decreased by −72%. The expression of osteoclast differentiation markers (NFATc1, TRAP, OSCAR) were reduced after sHA3 incubation and high glucose further decreased their expression while cell death events were diminished.

We showed that the collagen/sHA3 coating of TriLA scaffolds improved the defect filling in diabetic rats by supporting bone mineralization. Possibly, a decreased osteoclast differentiation due to osteogenic and anti-osteoclastic effects of osteoblasts may represent underlying mechanisms.

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Combination of novel two-photon photopolymerised scaffolds and bioactive elastin-like-recombinamers induce bone regeneration

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Non-healing fractures caused by trauma, disease or tumour resection demand the use of bone grafts to support and stimulate the healing process. We sought to develop and evaluate the effect of a novel bioactive biodegradable biomaterial designed to fill large bone lesions and to improve bone healing. We used two-photon polymerised synthetic polymer scaffolds composed of lactide (LA), caprolactone (CL), and methacrylate (MA) with varying LA: CL ratio and percentage of methacrylation. We tested 3 formulations: 1) LCM3 containing 8 LA: 2 CL and 85% MA; 2) LCM4 containing 9 LA: 1 CL and 90% MA and 3) LCM6.1 containing 9 LA: 1 CL and 40% MA which were either 2D polymer discs or 3D scaffolds and were combined with an injectable thermosensitive elastin-like-recombinamer (ELR) biogel containing the RGD cell adhesion domain and bone morphogenetic proteins (BMP2, BMP7), and hydroxyapatite nanoparticles (HA NPs). We evaluated *in vitro* mouse osteoblast (OB) and osteoclast (OC) differentiation and cell function and in vivo bone regeneration using a critical-sized calvarial defect (Ø=4 mm) in 8-week old female BALB/c mice. OC development and OB differentiation to mature mineralizing OBs were observed for all scaffold materials. Osteoblastogenesis was supported in the presence of ELRs and results suggest that BMP2 and BMP7-functionalized ELRs simulated OB differentiation. Combined analysis of Quantum FX microCT and histology revealed in vivo cellular attachment associated with osteoconduction. New bone formation was only observed when LCM3 was combined with BMP-containing ELRs. HA NPs enhanced bone regeneration when combined with ELRs and scaffolds. This innovative combination of biomaterials has an osteoinductive effect with potential clinical implications.

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In vitro investigation of the effect of Magnetic Resonance guided Focused Ultrasound Surgery on osteosarcoma cell lines

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Aim

Magnetic Resonance guided Focused Ultrasound Surgery (MRgFUS) is an invasive treatment able to control local disease and pain of bone tumors. Unfortunately, there is not any scientific evidence of the biological effect of MRgFUS treatment on tumor cells, especially in lower dose region, where tissues are only warmed to sub-lethal temperatures. Here we investigate the effect of *in vitro* MRgFUS treatment, at different levels of acoustic energy (200–630 J), on cell viability and osteogenic differentiation (ALPL, RUNX2, SPP1 and BGLAP genes expression) of osteosarcoma cell lines.

Material and methods

The Mg-63 and Saos-2 cell lines were exposed to the MRgFUS treatment (ExAblate 2000 system, In Sightec Ltd., Haifa, Israel) and analyzed for their viability and osteogenic differentiation at three different experimental times: 24 hours, 7 days and 14 days. The proliferation of osteosarcoma cell lines was evaluated by a CellTiter-Glo Luminescence Assay (Promega Fitchburg, WI, USA) whereas the relative gene expression was quantified through the comparative Ct method (StepOne Real-Time PCR System, Applied Biosystems).

Results

Cell viability assay of Mg-63 cell line exposed to MRgFUS showed a higher cell viability 14 days after treatment. On the contrary, the treatment of Saos-2 cell lines with focused ultrasound waves at low intensity (200–400 J) does not cause significant cell viability variation, comparing to the control group. These data were confirmed by the analysis of gene expression which, albeit with variations among the different experimental conditions, they do not seem to highlight a progression to cell's differentiation.

Conclusion
These data suggest that MRgFUS treatment at low intensity may induce cellular proliferation in vitro. Further analysis are necessary in order to better define, in the clinical practice, the area of ablative treatment and reduce the possibility of inducing cancer cell's proliferation.

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Increased periosteal expansion, Osterix expression and osteogenic potential upon bone injury during perturbed PI3K signaling

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Periosteum contains mesenchymal progenitors and is essential for fracture healing. Signaling mechanisms governing periosteal reaction to injury remain largely unidentified. We previously investigated how PI3 kinase signaling affects the skeletal system using $Cbl^{YF/YF}$ knock-in (YF) mice wherein PI3K signaling is perturbed by abolition of interaction between Cbl, an E3 ubiquitin-ligase/adaptor protein, and p85 subunit of PI3K. YF mice displayed increased bone volume under homeostatic conditions and formed larger bony callus during fracture repair. Given these data and because PI3K regulates osteoblast differentiation, we hypothesized that aberrant PI3K signaling regulates fracture healing by modulating osteogenic commitment of periosteal progenitors upon injury. To visualize periosteal cells, WT-$Osterix^{RFP}$ or YF-$Osterix^{RFP}$ mice were generated and used as per IACUC protocols. Creation of stabilized femoral fractures in these mice and comparative histological analyses at days 1 and 3 post fracture revealed no changes in intact femoral periosteum. Interestingly, Osx^{RFP+} periosteal thickness in YF-Osx^{RFP} mice was significantly increased over WT-Osx^{RFP} in D1 fractured femurs (WT-28 μm, YF-57 μm, $P<0.05$, $n=3$) and D3 fractured femurs (WT-90 μm,YF-189 μm, $P<0.05$, $n=6$). Osx^{RFP+} periosteal cells in D3 fractured femurs were 1.6-fold higher in YF-Osx^{RFP} over WT-Osx^{RFP}. Osteogenic potential of injured periosteum was detected by alkaline phosphatase staining. ALP^+ periosteum was increased in D1 fractured femurs (WT-35 μm, YF-50 μm, $n=3$) but showed threefold enhancement in D3 fractured femurs in YF-Osx^{RFP} over WT-Osx^{RFP} (WT-58 μm, YF-170 μm, $P<0.05$, $n=6$). Since PI3K signaling regulates cell proliferation, we examined periosteal proliferation upon injury by EdU labeling. 3 days post fracture, significantly more EdU^+ periosteum was observed in YF-Osx^{RFP} than WT-Osx^{RFP} (WT-4%, YF-22%, $P<0.05$; $n=3$). Flow cytometric analysis of isolated periosteal cells revealed that in YF mice, fractures led to enhanced expression of MSC markers, Sca1 (Intact-5.5%, Fx-13.5%, $P<0.05$), CD29 (Intact-9.6%, Fx-20%, $P<0.05$) and CD105 (Intact-6.6%, Fx-12.1%, $P<0.05$) over intact femurs with no significant changes seen in WT ($n=4$). In conclusion, perturbed PI3K signaling, upon injury, promotes expanded, highly proliferative, $Osterix^+$ periosteum with more MSC-like cells and enhanced osteogenic potential.

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Multicentre study reveals poor correlation between *in vitro* and *in vivo* assessments of biomaterials for bone-regeneration

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Introduction
Research on biomaterials for bone regeneration generates a plethora of new biomaterials requiring evaluation with reliable and comparable methods of biocompatibility and functionality for clinical translation. To reduce the burden of *in vivo* assessment, there is a need for refined *in vitro* assays that are predictive of *in vivo* outcomes. This retrospective study correlated *in vitro* results with *in vivo* outcomes observed for a range of biomaterials for bone regeneration.
Methods
Members from eight universities in the European consortium BioDesign kindly provided the data. Assessors from each lab scored both *in vitro* and *in vivo* outcomes of individual biomaterial experimental variables (1 = poor to 5 = very good) on the basis of assessor-defined criteria particular to each assay. The data included 36 *in vivo* studies 47 *in vitro* assays and 93 materials. Data were sorted into sub-groups of different *in vitro* assays. In addition, *in vitro* assays were combined in pairs to investigate if combinations of *in vitro* assays allowed better prediction of *in vivo* outcomes compared to single assays.
Results
There was no significant overall correlation between *in vitro* and *in vivo* outcome. The mean *in vitro* scores shared 58% of variance to the *in vivo* scores. The mean *in vivo* scores shared 51% of variance to the *in vitro* scores. All combinations of *in vitro* assays showed less than 10% covariance, except for one combination of alkaline phosphatase and biocompatibility assays, which shared 95% covariance though low statistical power limits the conclusions that can be drawn.
Conclusion
The conventional biomaterial-testing pipeline is susceptible to selection bias through a tendency to select only materials with positive *in vitro* outcomes for testing in subsequent *in vivo* studies. This makes the poor correlation surprising. Current findings encourage the development of novel approaches of biomaterial assessment able to reliably predict *in vivo* outcomes.

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

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

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Bone Marrow

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Abstract withdrawn.

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P79

Bone marrow adipose tissue and bone turnover in postmenopausal osteoporotic women and the effects of raloxifene

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Background
In postmenopausal osteoporosis, a loss of bone volume due to increased bone turnover is accompanied by a higher volume of bone marrow adipose tissue (BMAT). If this static relationship is based on a functional relationship, BMAT is a potential target for treating osteoporosis. While it is known that estrogen can reduce BMAT, it is still unknown whether raloxifene – a selective estrogen receptor modulator – can also reduce BMAT.
Objective
To determine 1) the correlation between BMAT and bone turnover in postmenopausal osteoporotic women and 2) the effect of raloxifene on BMAT.
Methods
We retrospectively analyzed paired iliac crest biopsies from 26 postmenopausal osteoporotic women enrolled in the MORE trial, both at baseline and after 2 years of treatment with placebo or raloxifene. All subjects received oral calcium and vitamin D3. Standardized bone histomorphometry and BMAT parameters were measured in Goldner stained sections.
Results
At baseline, BMAT was correlated with bone volume ($R=-0.382$; $P=0.03$), but not with bone formation rate or osteoclast number. There was an increase in adipocyte density in the raloxifene group, while there was no change in adipocyte density in the in the placebo group (mean change from baseline: 34.1 s.d. 28.9 *vs* −3.5 s.d. 29.5 cells/mm^2; $P=0.003$). The adipocyte diameter did not change after raloxifene, while after placebo there was an increase in adipocyte diameter (mean change from baseline: −0.5 s.d. 2.8 *vs* 3.1 s.d. 5.1 μm; $P=0.03$).
Conclusion
This study suggests that BMAT is not correlated with bone turnover in iliac crest biopsies of postmenopausal osteoporotic women. Furthermore our results indicate that raloxifene does not reduce the volume of BMAT, but rather increases the number of bone marrow adipocytes while preventing an increase in the size of bone marrow adipocytes.

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Multi-potency and immunosuppressive activity of mesenchymal stromal cells derived from human induced pluripotent stem cells

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Tissue healing/reconstruction as well as exacerbated inflammatory diseases may benefit from stem cell based therapies. *Ex vivo* isolated tissue mesenchymal stromal cells (MSCs) displaying multi-potent activity and immune-regulatory functions were long ago proposed as therapeutic cells and already tested in many clinical assays. Nevertheless, their use may be restricted because of the few number that can be recovered from adult tissues, their limited *in vitro* expansion, and the absence of a full characterization. Other sources of well-defined and unlimited number of MSCs are needed, and MSCs derived *in vitro* from human Induced Pluripotent Stem (huIPS) cells would be a valuable tool for therapeutic approaches.
We developed a simple assay to generate huiPS-MSCs which were evaluated for their multi-potency and their immunosuppressive activity *in vitro* and *in vivo* in a humanized mouse model.
1) The huiPS-MSCs were phenotypically indistinguishable from tissue MSCs; they were capable of differentiation into osteoblasts, chondrocytes and adipocytes. Co-cultured with stimulated human T lymphocytes, huiPS-MSCs inhibited efficiently the T cell proliferation, switching the T cell cytokine polarization to a regulatory state.
2) The *in vivo* immunosuppressive activity of huiPS-MSCs was evaluated using immune-deficient NOD/SCID/IL2rγKO mice in which human immune cells proliferate, infiltrating tissues. After treatment with huiPS-MSCs, the numbers of human circulating T lymphocytes, of those present in the peritoneal cavity and in the spleen were significantly reduced. Intra-cytoplasmic labelling of recovered T cells showed that untreated mice displayed high percentages of T cells producing inflammatory IFN and TNF cytokines. In contrast, in mice treated with the huiPS-MSCs, the proportion of inflammatory T cells was reduced, while that of T cells producing the anti-inflammatory IL-10 cytokine and expressing the FoxP3 was significantly increased.
We show that *in vitro* generated multi-potent immune-modulatory huiPS-MSCs may served as new therapeutic tolerogenic tools for inflammatory diseases or for reconstruction/healing processes.

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P81

Increased bone marrow adiposity in energy deficit context: tip of the iceberg?

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Context
An increase in bone marrow adiposity (BMA) is usually described in anorexia nervosa (AN) patients and in calorie restriction models. This induced BMA could be involved in the development of the osteoporosis often described in AN. However, BMA increase is not always observed and could be depending on the severity of the deficit.
We previously developed the separation-based anorexia mouse model (SBA)[1] (Ethical approval CEEA #022012). SBA mice displayed a severe body weight loss and a low bone mass, but no obvious increase in BMA.
Objectives
To determine the potential association between body weight loss, bone mass and BMA in a calorie deficit context.
Methods
The SBA model was adapted to target various levels of body weight loss. At the end of the 10 week protocol, we assessed tibia and vertebral bone parameters using microCT. We determined changes in volume and location of BMA after osmium staining. The capability of bone marrow stromal cells (BMSCs) to differentiate in adipocytes and osteoblasts was also determined in our co-differentiation medium[2].
Results
First results showed that bone alterations take place without increase in BMA. But, 48 h after plating, PPARγ expression was only found in BMSCs from SBA mice. BMSCS from each SBA group displayed an increase in the adipocyte differentiation capabilities at the expense of osteoblastic differentiation. Lipid storage in adipocytes was detected as soon as 3 days after induction of differentiation *vs* 10 days for BMSCs from control mice. The expression of adipocyte markers was increased and that of the osteoblastic markers reduced in cells from SBA mice.

Conclusions
These results suggest that BMA could be a late marker of early changes responsible for alterations of bone phenotype.
1 Zgheib S, et al., 2014 *PLoS ONE* 9(8): e103775.
2 Ghali O, et al., 2015. *BMC Cell Biol* 16:9, DOI 10.1186/s12860-015-0056-6.
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Bone Matrix

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CRISPR/Cas9-mediated IFITM5 gene editing demonstrates that BRIL (Ser40Leu) substitution suppresses PEDF-mediated activation of PPARγ

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Osteogenesis imperfecta (OI) type VI is caused by recessive null mutations in *SERPINF1*, encoding pigment epithelium-derived factor (PEDF), an anti-angiogenic secretory glycoprotein. Dominant mutations in *IFITM5*, encoding BRIL (*B*one *R*estricted *I*fitm-*L*ike), a transmembrane protein upregulated in osteoblasts during mineralization, cause either type V OI (c.-14C>T, addition of 5 amino acids on BRIL) or atypical type VI OI (c.119C>T, p.Ser40Leu substitution in BRIL intracellular domain). Atypical type VI OI patients have extremely severe bone dysplasia, histological features of type VI OI bone, but no characteristics of type V OI. We reported that type V OI *IFITM5* mutations cause an increased SERPINF1 expression and PEDF secretion in proband osteoblasts, while patient osteoblasts with the Ser40Leu mutation display decreased *SERPINF1* expression and PEDF secretion. Currently, little is known about how point mutations in BRIL impact *SERPINF1* expression. Interestingly, overexpression of BRIL (Ser40Leu) in mesenchymal stem cells did not impact on PEDF secretion, which was significantly decreased in the osteoblasts from the atypical OI type VI patient. To further investigate the underlying mechanism by which BRIL regulates PEDF secretion and thereby osteoblast matrix mineralization, we generated a cellular system that carries the *IFITM5* (c.119C>T) mutation, using the CRISPR/Cas9-mediated gene editing system. We designed a donor DNA for homology-directed repair-mediated gene modification and obtained several clones that harbor the heterozygous *IFITM5* (c.119C>T) mutation. In CRISPR/Cas9-edited clones, significantly decreased *SERPINF1* expression and PEFD secretion recapitulated our previous observation with cells from the atypical OI type VI proband. Moreover, the *IFITM5* (c.119C>T) mutation resulted in an decreased expression of PPARγ, also seen in osteoblasts from the *serpinf1*-null mice. These results demonstrate that BRIL-mediated induction of PEDF activates PPARγ-mediated cellular events. This study also demonstrated that CRISPR/Cas9-mediated gene editing can be a useful tool to simulate cellular status and to study molecular mechanisms of OI-causing mutations.

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Cortical bone matrix mineralisation is decreased in TRPV4 deficient male, but not in female mice

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Transient receptor potential vanilloid channels (TRPVs) have been implicated in Ca2+ homeostasis and bone metabolism. In particular, TRPV4 deficiency was shown to cause sexual dimorphism in bone metabolism and osteoporotic fracture risk. Cortical bone structure was reported to be altered in male TRPV4 knock-out (TRPV4−/−) mice but not in female TRPV−/− mice compared to sex-matched wildtype (TRPV4+/+) animals.
To gain knowledge on this sexual dimorphism, we studied the bone matrix mineralization density distribution (BMDD) based on quantitative backscatter electron imaging in cancellous and cortical femoral bone from six male and six female TRPV4−/− mice as well as from six male and six female TRPV4+/+ mice.
The results from trabecular metaphyseal (MS) and epiphyseal (ES) femoral bone showed that both TRPV4−/− male and TRPV4−/− female mice had a shift of their BMDD to lower calcium concentrations compared to sex-matched TRPV4+/+ animal groups. The typical degree of mineralization CaPeak (the peak position of the BMDD) was reduced in both sexes: MSCaPeak was decreased by −6.3% ($P<0.001$) and −4.3% ($P<0.05$) and ESCaPeak by −7.2 and −5.4% (both $P<0.01$) for male and female mice respectively. In contrast, in diaphyseal cortical (CT) bone, only the male TRPV4−/− revealed lower CTCaPeak −5.8% ($P<0.01$) compared to male TRPV4+/+ while in the female mice CTCaPeak was not affected by the TRPV4 genotype.
These BMDD findings are a further indication for sexual dimorphism by the TRPV4 genotype. In particular, cortical bone properties including mechanical performance, structure and mineralization are differentially affected in male and female TRPV4−/− mice.

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Anabolic parathyroid hormone (PTH) treatment does not alter periosteal bone mineral composition during primary and secondary mineralisation

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Parathyroid hormone (PTH) is used to stimulate bone formation in osteoporotic patients, however concerns have been raised about the quality of the matrix produced since lower levels of total matrix mineral have been reported in osteoporotic and fracture patients treated with PTH. High resolution synchrotron-based Fourier Transform Infrared Microscopy (sFTIRM) was used to determine mineral content in age-matched bone during anabolic PTH treatment, using the simplified lamellar structure of growing murine cortical bone.
Eight week old male mice were treated with vehicle or 50 μg/kg PTH, 5 times/week for 4 weeks ($n=7$–9/group). Histomorphometry and 3-point bending tests confirmed increased trabecular and periosteal bone formation and increased femoral strength in PTH treated mice. Dual calcein labels allowed region-based analysis by sFTIRM at the midshaft, in six 15 μm^2 regions perpendicular to the most immature bone on the periosteal edge of the medial cortex in the same regions where increased bone formation was measured by histomorphometry. All animal procedures were conducted with approval from the St. Vincent's Health Melbourne Animal Ethics Committee.
This sFTIRM method was validated in control bones by detecting the expected progressive increase in mineral:matrix ratio and collagen crosslink content from the periosteal edge (new) to the inner (mature) bone. PTH treatment did not alter the progressive changes in these parameters, nor did it alter crystallinity or carbonate content, within the maturing bone, indicating that bone deposited during PTH treatment has the same composition as age-matched bone deposited during normal periosteal bone growth.
These data confirm that earlier studies detecting lower levels of mineralisation in PTH-treated bone reflected a higher proportion of newly formed bone during modelling, rather than a change in collagen maturation and mineral accrual during PTH treatment.

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MicroRNA-125b in bone matrix plays a crucial role in osteoblast-osteoclast communication

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Matrix vesicles (MVs) play a key role in bone mineralization. These nano-sized vesicles bud from apical microvilli of osteoblasts and accumulate in osteoid, and initial hydroxyapatite crystals form within these vesicles. MVs appear to share a common feature with exosomes. Recent studies have demonstrated that exosomes include microRNAs (miRNAs) as mediators of intercellular communication. We purified MVs from MC3T3-E1 cell cultures by ultracentrifugation and found approximately 200 miRNAs. MVs suppressed RANKL-dependent osteoclast formation and bone resorption in mouse bone marrow macrophage (BMM) and/or RAW-D cell cultures with decreased levels of osteoclast marker genes such as *Acp5*, *Ctsk* and *Dcstamp*. There were no obvious effects of MVs on rat and human osteoblast and MC3T3-E1 cell cultures. We then focused on miRNAs with predicted target genes involved in osteoclast formation. In both human and rodent models, miR-125b was highly abundant in MVs, compared to osteoblasts and BMMs. Moreover, we found that miR-125b was complexed with Ago2 and stored in the extracellular matrix of MC3T3-E1 cell cultures and mouse bone. Besides being a suppression of osteoclast formation, miR-125b decreased the expression of osteoclast marker genes. The transcriptional repressor Prdm1 was down-regulated by miR-125b, resulting in increases in the expression of the anti-osteoclastogenic genes, *Irf8* and *Mafb*. To determine whether miR-125b affects bone *in vivo*, we generated transgenic mice overexpressing miR-125b under the control of the human osteocalcin promoter (Tg). Overexpressing miR-125b in osteoblasts in Tg mice led to high bone mass by decreasing the number of osteoclasts without any changes in osteoblasts. miR-125b suppressed osteoclastic osteolysis in the lipopolysaccharide -induced mouse osteolysis model. These findings suggest an additional role of MVs and that miR-125b embedded in bone regulates osteoclastogenesis, which may serve as a novel mechanism for osteoblast-osteoclast communication.

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Calciotropic and phosphotropic hormones and mineral metabolism

P86

Possible paracrine effects of fibroblast growth factor-23 in the regulation of intestinal calcium absorption

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As a bone-derived hormone that regulates phosphate homeostasis and renal 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$] production, fibroblast growth factor (FGF)-23 has been postulated to indirectly control calcium and bone metabolism by modulating the levels of phosphate and $1,25(OH)_2D_3$. However, the presence of FGF receptor-1 (FGFR-1) proteins in the intestinal epithelial cells suggests that the intestine may directly respond to FGF-23. The present study, therefore, aimed to investigate the local production of FGF-23 as well as its paracrine actions in the intestine. The expression of FGF-23 proteins in the rat small and large intestine was determined by quantitative immunohistochemistry. In early lactating rats with high calcium demand for lactogenesis (day 8 of lactation), the FGF-23 levels in the duodenum and cecum were found to markedly increase. This phenomenon was hypothesized to prevent excessive calcium absorption. In addition, the lactation-induced upregulation of intestinal FGF-23 expression was diminished by 7-day s.c. injection of bromocriptine, a potent inhibitor of pituitary release of prolactin that is one of the calcium-regulating hormones during lactation. Similar findings were also observed in late lactating rats (day 21 of lactation). An *in vitro* radioactive calcium flux study in intestinal epithelium-like Caco-2 monolayers showed that FGF-23 was capable of inhibiting the prolactin-enhanced transepithelial calcium transport. Our results thus corroborate the possible paracrine effects of FGF-23 in the rat intestine, especially during lactation, which may help counterbalance the actions of calciotropic hormones, and prevent excessive calcium absorption.

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P87

Reciprocal correlation between iron and calcium transport and the hepcidin-enhanced calcium absorption in the duodenum of hemizygous beta-globin knockout thalassemic mice

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A decrease or absence of beta-globin production in erythroid cells causes a hereditary anemic disorder known as beta-thalassemia, which is associated with ineffective erythropoiesis, iron overload, growth retardation, osteopenia, and osteoporosis. Our recent investigation revealed that beta-thalassemia led to osteoclast-mediated bone resorption and the impaired intestinal calcium absorption, the latter of which modestly responded to conventional 1,25-dihydroxyvitamin D_3 supplementation. In the present study, hemizygous beta-globin knockout thalassemic mice were used to investigate how to rescue calcium absorption. The study has been approved by institutional animal care and use committee. The results showed that transepithelial calcium flux across the duodenal epithelia of thalassemic mice was inversely correlated with the iron flux, as determined by ^{45}Ca and ^{59}Fe radioactive tracers in Ussing chamber studies. In addition to changes in iron and calcium transport, thalassemic mice also exhibited a lower paracellular flux of zinc as compared to the wild-type littermates. Iron hyperabsorption in beta-thalassemic mice probably resulted from overexpression of apical iron transporters, particularly divalent metal transporter (DMT)-1, which was apparently prevented by a liver-derived hormone hepcidin. Direct exposure to hepcidin in Ussing chamber led to downregulation of DMT1 protein expression and inhibition of transepithelial iron transport concurrently with stimulation of calcium absorption. The hepcidin-induced transcellular calcium transport was markedly diminished by inhibitors of Na^+/Ca^{2+} exchanger 1 and plasma membrane Ca^{2+}-ATPase, suggesting that hepcidin predominantly modulated the transcellular calcium transport rather paracellular transport. In conclusion, beta-thalassemia leads to calcium malabsorption and iron hyperabsorption, both of which can be rescued by hepcidin administration.

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Trabecular (Spine) bone density increases significantly in the first 6 months after weaning (Factors Affecting Bone Formation After Breastfeeding Pilot Study (FABB-Pilot))

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Breastfeeding women resorb trabecular bone to supply much of the calcium content of milk. Few studies have examined the speed and extent of BMD recovery after weaning, or the factors that predict a greater post-lactation increase in BMD. We hypothesize that weight-bearing, nutrition, hormones, and other factors facilitate bone formation after lactation.

The aims of the Factors Affecting Bone Formation After Breastfeeding Pilot Study (FABB-Pilot) were to determine the magnitude of increase in BMD at 6 months after weaning, and provide preliminary data to enable pre-specification of predictive variables to be tested in a future larger-scale FABB study.

We recruited women who had breastfed exclusively (no formula) or near-exclusively (1–2 formula/solid feeds per day) for 4–6 months, and by so doing should have experienced a significant decline in BMD. At time of planned weaning and 6 months later, we measured BMD, whole body fat and lean mass, and hip structural analysis by DXA; blood calcium, ionized calcium, PTH, PTHrP, estradiol, 25OHD, calcitriol, P1NP, CTX; and urine Ca/Cr. Questionnaires administered at both time points assessed nutrition, weight-bearing activities, and other factors that may influence bone recovery.

31 women (31.6±3.5 years, 97% Caucasian) enrolled at 26±2.0 weeks postpartum and completed the baseline measurements. Approximately 80% had breastfed exclusively for 6 months. 30 women completed the follow-up assessments. Mean thoracic spine BMD increased 5.1% (0.713–0.749 g/cm^2, $P<0.01$); lumbar spine increased 4.0% (0.971–1.01 g/cm^2, $P<0.03$), while cortical sites (hip, total body) remained unchanged. Estradiol increased (115–198 pmol/l, $P<0.01$), PTH increased (60–69 ng/ml, $P<0.04$), and 25OHD declined (80 vs 64 nmol/l, $P<0.01$), while calcium and ionized calcium did not change.
In conclusion, trabecular (spine) BMD increases significantly in the first 6 months post-weaning accompanied by increased estradiol and PTH. The factors that promote this post-lactation increase in BMD remain to be identified.

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Endogenous expression and biological functionality of secreted Klotho in human mesenchymal stem cells

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The biological relevance of Klotho in aging processes was impressively demonstrated in transgene and knock out mouse experiments and its impact on calcium, vitamin D and phosphate metabolism suggests multifunctional modes of action.
The human alpha Klotho gene encodes two transcripts, one for a protein of 130 kDa with a short intracellular and a transmembrane region and the two extracellular domains KL1 and KL2. A second transcript exists due to alternative splicing, encoding a secreted protein of 70 kDa with sequence identity to KL1, but differing in the last 15 amino acid residues. Beyond that, the transmembrane region can be cleaved by metalloproteinases, generating a circulating Klotho protein whose domains KL1 and KL2 can furthermore be proteolytically separated. This does not only multiply the modes of action of Klotho, the high sequence identity makes it also difficult to analyze differences between the two transcript variants. Specific antibodies, which are able to distinguish between the KL1 domains, generated by proteolytic cleavage or by alternative splicing, are necessarily required. As yet it is unknown if specific receptors exist to transduce biologic effects of the KL1 domains.
We recently established a stable cell line expressing the alternatively spliced KL1 domain and analyzed its effect on human mesenchymal stem cells (hMSC), alone and in combination with FGF23. Furthermore, we characterized a new antibody generated by Immundiagnostik AG, which is specific for this Klotho variant. This enabled us to analyze endogenous expression and intracellular localization of Klotho in hMSC.
Immunocytochemical analyses of HEK293 cells transfected with expression plasmids coding for membrane bound or secreted Klotho, respectively, clearly demonstrated the specificity of the new antibody. We could detect endogenous expression of this Klotho variant in hMSC by RT-PCR and immunocytochemistry, however donor-dependent differences were observed. Stimulation of hMSC with secreted Klotho can induce expression of egr1, not only in combination with FGF23, even admitted alone. Furthermore, stimulation with labeled secreted Klotho suggests an uptake of extracellular Klotho into the cell, but the mechanism remains unknown.
These observations presume an autocrine/paracrine mechanism in hMSC, possibly with impact on the wnt-pathway, since slight induction of connexin 43 was measured in qPCR.

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P90

Bone loss in KLHL3 knock-in mice characterized by a pseudohypoaldosteronism type II-like phenotype is mediated by renal PTH resistance

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Pseudohypoaldosteronism type II (PHAII) is a hereditary disease characterized by hypertension, hypercalciuria and osteopenia. PHAII is caused by mutations in with-no-lysine kinase 1 (WNK1), WNK4, or the cullin RING ligase family members kelch-like 3 (KLHL3) or cullin 3 (CUL3). All mutations result in up-regulation of the WNK signalling pathway which activates thiazide-sensitive Na-Cl cotransporters (NCC) in renal distal tubules, leading to salt retention and hypertension in PHAII. The mechanism underlying hypercalciuria in PHAII is unknown. To better understand the mechanisms leading to osteopenia in PHAII, we used KLHL3R528H/+ knock-in mice carrying the same mutation as some PHAII patients. As expected, KLHL3R528H/+ mutants exhibited hyperkalemia, hypernatremia, renal calcium wasting and increased phosphorylation of NCC in the kidney. Furthermore, KLHL3R528H/+ mutants showed elevated serum parathyroid hormone (PTH), increased bone resorption as demonstrated by elevated urinary collagen crosslinks excretion and increased osteoclast numbers in femoral cancellous bone, and reduced distal femoral cancellous bone BMD and volume as evidenced by pQCT and μCT analysis. Analysis of the expression of proteins involved in renal calcium transport revealed elevated membrane abundance of the fully glycosylated epithelial calcium channel TRPV5, decreased TRPV6 abundance, and unchanged calbindin D28k expression in KLHL3R528H/+ mutants. In contrast to the upregulated TRPV5 protein expression, TRPV5 phosphorylation was reduced in KLHL3R528H/+ mutants, suggesting downregulated TRPV5 activity. In line with a crosstalk between NCC activity and PTH-mediated TRPV5 activation, we found by 2-photon microscopy that the PTH-mediated increase in Ca^{2+} uptake in mouse distal tubular mpkDCT4 cells was enhanced by the NCC blocker chlorothiazide or by knockout of NCC. Taken together, our study provides a mechanistic explanation for the hypercalciuria and bone loss found in PHAII patients: elevated NCC activity in KLHL3R528H/+ mice blocks PTH-mediated TRPV5 activation, leading to renal PTH resistance with subsequent renal Ca wasting and a counter-regulatory PTH-induced bone loss.

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PTH and vitamin D in Inuit and non-Inuit in Greenland

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Background
Sun exposure may be limited in Arctic populations and Greenland Inuit have adapted to the Arctic environment. The influence of chronic low vitamin D level on PTH to uphold a normal serum calcium remains to be elucidated.
Objective
To describe the association between PTH and factors known to influence PTH-, including vitamin D (25OHD) and calcium, in Arctic populations.
Methods
Inuit and non-Inuit in the capital city Nuuk in West Greenland and in the major town and in remote settlements in East Greenland were surveyed. Supplement use and life-style factors were determined by questionnaires, dietary habits by a food frequency questionnaire, and PTH, calcium and 25OHD were measured in serum.
Results
One percent of the population of Greenland was invited and 95% participated with 434 Inuit and 101 non-Inuit. Serum 25OHD was below 50(20) nmol/l in 23.0(0.2)% Inuit and 70.9(13.9)% of non-Inuit. Median serum PTH was 2.3(1.5) u/l in Inuit and 2.8(4.6) u/l in non-Inuit with plasma 25OHD below 50(20) nmol/l. Inuit and non-Inuit had different levels of serum calcium ($P=0.023$), PTH ($P<0.001$) and 25OHD ($P<0.001$). Factors important to PTH in multivariate regression analysis were 25OHD ($P=0.007$) and ethnicity ($P<0.001$).
Conclusions
Greenland Inuit had a lower PTH for the same level of vitamin D in serum compared with non-Inuit. This suggests that Arctic Inuit may have adapted to a low 25OHD status.

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The analysis of missed diagnosis and misdiagnosis of 144 tumor-induced osteomalacia patients

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Introduction
Tumor-induced osteomalacia (TIO) is a rare acquired paraneoplastic syndrome which is usually induced by mesenchymal tissue tumor with excessive secretion of FGF23. The misdiagnosis of TIO is frequently seen in clinic. Therefore, this study is aimed to describe the misdiagnosed situation of TIO, explore the possible underlying reasons for missed diagnosis and misdiagnosis through the analysis of 144 TIO patients, and improve clinicians' awareness of TIO.
Methods
The clinical data of 144 surgically treated TIO patients in our hospital during December 1982–December 2014 were retrospectively analysed, including general information, clinical manifestation, laboratory examination, missed diagnosis and misdiagnosis.
Results
1. General Information. There were 80 male cases (55.6%) and 64 female cases (44.4%), with the onset age being 37.5 ± 11.4 years old. The pathological type was mostly phosphate urinary mesenchymal tumor. After resection of the responsible neoplasm, 117 cases achieved serum phosphorus recovery. 2. The clinical manifestations of TIO mainly included bone pain (99%), difficulty in activity (93%), non-violent fractures (80%), muscle weakness (65%), shorter height (69%), thoracic deformity (33%) and spinal deformity (27%). Bone pain might be the initial symptom (92%). 3. Patients demonstrated low serum phosphorus (0.48 ± 0.13 mmol/l), decreased tubular maximum of phosphate/glomerular filtration rate (TMP/GFR)(0.40 ± 0.17), elevated serum alkaline phosphatase (282.6 ± 161.0 U/l). 4. Missed diagnosis and misdiagnosis analysis. The clinic departments TIO patients referred to mainly distributed in endocrinology (35%), orthopedics (26%), rheumatology (23%), neurology (8%). The misdiagnosis rate was 95%. TIO was frequently misdiagnosed as intervertebral disc herniation (46 case-time), spinal arthritis (including ankylosing spondylitis) (38 case-time), osteoporosis (37 case-time), and other diseases including arthritis, femoral head necrosis, hyperparathyroidism, etc. The missed diagnosis rate of hypophosphatemia was high (43%).
Conclusions
TIO is a vital cause of adult-onset hypophosphatemia in China, but clinicians generally lack awareness and knowledge of this disease. Consequently, the misdiagnosed rate of TIO is high. Therefore, clinicians should further enhance their recognition of TIO and pay attention to searching for tumor in adult-onset patients with hypophosphatemic osteomalacia.

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Constitutively elevated blood serotonin is associated with bone loss and type 2 diabetes in rats

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Reduced peripheral serotonin (5HT) in mice lacking tryptophan hydroxylase (TPH1), the rate limiting enzyme for 5HT synthesis, was reported to be anabolic to the skeleton. However, in other studies, TPH1 deletion either had no bone effect or an age-dependent inhibition of osteoclastic bone resorption. The role of 5HT in bone therefore remains poorly understood. To address this issue, we used selective breeding to create rat sublines with constitutively high (high-5HT) and low (low-5HT) platelet 5HT level (PSL) and platelet 5HT uptake (PSU). High-5HT rats had decreased bone volume due to increased bone turnover characterized by increased bone formation and mineral apposition rate, increased osteoclast number and serum C-telopeptide level. High-5HT animals also developed a type 2 diabetes (T2D) phenotype with increased: plasma insulin, glucose, hemoglobin A1c, body weight, visceral fat, β-cell pancreatic islets size, serum cholesterol, and decreased muscle strength. Serum calcium accretion mediated by parathyroid hormone slightly increased, whereas treatment with $1,25(OH)_2D_3$ decreased PSL. Insulin reduction was paralleled by a drop in PSL in high-5HT rats. *In vitro*, insulin and 5HT synergistically up-regulated osteoblast differentiation isolated from high-5HT rats. TPH1 inhibition *in vitro* decreased the number of bone marrow-derived osteoclasts, while *in vivo* daily systemic administration of the TPH1 inhibitor (LX 1032) for 6 weeks reduced PSL and increased the trabecular bone volume and number of spine and femur in high-5HT rats. These results suggest that constitutively elevated PSL is associated with bone loss and T2D via a homeostatic interplay between the peripheral 5HT, bone and insulin.

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Automated ELISA for direct measurement of free 25OH vitamin D

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Recent studies suggest that the concentration and genotype of vitamin D binding protein are important factors that determine the bioavailability of 25OH Vit-D in blood. It has been suggested that measurement of free, non-protein bound 25OH Vit-D in serum, may provide more relevant diagnostic information than total 25OH Vit-D, for instance in chronic kidney disease, bladder cancer and pancreatic cancer, or in hemodialysis patients.
To measure free 25OH Vit-D in blood, Future Diagnostics developed a direct ELISA method. Following the first laboratory evaluation phase, a two-step enzyme-linked immunosorbent assay (ELISA) was optimized for the quantification of free 25OH Vit-D assay. Modifications were made in the protocol for the coating of the monoclonal anti-25OH Vit-D in the microtiter plates as well as in the formulation of the sample diluent and of the biotinylated vitamin D conjugate. The optimized assay was validated against the first version of the assay and showed the following performances: the calibrator range was 0.2–35 pg/ml. The LoB was 1.9 pg/ml; the LoD was 2.8 pg/ml. Total assay precision was 10.2% at 6.0 pg/ml, 7.6% at 10.9 pg/ml and 5.5% at 24.9 pg/ml. The cross-reactivity of the antibody towards 25OH vitamin D2 was 77% and the influence of interfering hemoglobin, bilirubin and triglycerides was also verified.
The free 25OH Vit-D assay that reproducibly determines the level of free 25OH Vit D in serum was implemented and validated on an open ELISA platform. After adaptation of the assay protocol on the instrument, manual and automated results were compared in terms of dose response curve, accuracy, precision, sensitivity and drift.
This assay can be used as a valuable tool in studies to establish the clinical relevance of free 25OH Vit-D.

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Relationship between risk of preterm birth and vitamin D deficiency

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Background and objectives
Vitamin D deficiency causes calcium-depleted bone, which further weakens the bones and increases the risk of fractures. Also, It is known as related to obstetrics problem like gestational diabetes, infections, preeclampsia and incidence of caesarean section as well as glucose metabolism, immune system, cardiovascular diseases and cancer.
Recently, the relationship between preterm birth and vitamin D deficiency becomes the main interest and many studies have been done. We also planned to research the level of vitamin D in pregnancy women and find a relation with preterm labor to prevent preterm labor and do the active antenatal care.
Method
Study was done with 160 pregnancy women who taken vitamin D test (tandem mass) in our hospital from March 2013 to June 2014 checking the pregnancy outcome and medical history using the medical record.
Results
In this result, 21.3% were vitamin D-sufficient level during pregnancy. We cannot find statistical relation in preterm labor, gestational diabetes, pregnancy-induced hypertension, preeclampsia and the rate of caesarean section.
Conclusions
Following this study, we find many pregnancy women are lack of vitamin D and further study related to the role of vitamin D in obstetrics has to be made and more concerns are needed.

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Clinical characterization and genetic analysis of TRPV4-related skeletal dysplasias in 4 Chinese families

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TRPV4-associated skeletal dysplasias include: FDAB, ADBO, SMDK, SEDM, MD and Parastremmatic dysplasia. In this study, we recruited 3 families with congenital scoliosis and 1 family with localized digital osteopetrosis. We collected their clinical data and use the next-generation sequencing system, Sanger sequencing and RT-PCR to obtain the genetic diagnosis. Proband 1, 2, 3 all presented with early-onset kyphoscoliosis and short stature. X-ray showed platyspondyly, hemivertebra, accompanied by metaphyseal abnormalities. P1 also presented with waddling gait, bilateral streblomicrodactyly, P2 with bended upper arm and genu valgum, P3 with left femoral head subluxation. Bone turnover markers were normal. P4 presenteed with right phalanges thickening after frostbite. P4 and her mother also presented with shortening of the toes and teeth loss. X-ray showed cortical bone thickening. Two mutations of TRPV4 have been identified: Proband 1, 2, 3 were all heterozygous for c.1781G > A, a hot-spot mutation of SMDK. Proband 4 and her mother were heterozygous for a novel splice-site mutation c.1491 + 1G > A, and was proved to lead to skipping of exon 8 in the transcript. We firstly reported the SMDK and FDAB cases in the Chinese population. The three SMDK families carrying the same missense mutation R594H implicated that R594H may be a hot-spot mutation in Chinese. The novel splice-site mutation found in the FDAB family, is the first reported splice-site mutation of TRPV4 gene.

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Vitamin D therapy: the effects of race, skin colour and genotype

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Background
It is well established that Asians have lower levels of vitamin D (25OHD) when compared to Caucasians. Vitamin D binding protein (DBP) levels are also lower in some races than in Caucasians.
Objectives
i) To find out whether DBP is lower in Asians; ii) To find out whether free 25OHD is lower in Asians; iii) To find out whether PTH is increased in Asians and interpret this in relation to 25OHD levels and iv) To find out whether the increment in 25OHD levels is the same in Asians.
Methods
Prospective single centre clinical trial involving sixty healthy men (18–25 years): Caucasians ($n = 30$) and Asians ($n = 30$). Fasting serum samples were collected immediately before and 4 weeks after vitamin D_3 (150,000 IU) administration. We measured total 25OHD, free 25OHD (Future Diagnostics), albumin, DBP (Genways), DBP genotype, and we calculated free 25OHD.
Results
All subjects achieved a > 25 nmol/l increment in total 25OHD level following vitamin D administration (Table 1). At baseline, Asians had significantly lower serum total 25OHD and DBP levels, similar serum and calculated free 25OHD and higher PTH levels compared to Caucasians. Four weeks after supplementation, increment in serum total 25OHD was not different between the two groups however the increment in free 25OHD appears to be higher in Asians.

Table 1 Results showing serum total and free 25OHD levels, calculated 25OHD and PTH levels at baseline and increment post supplementation, mean and SD.

		Serum Total 25OHD (nmol/l)	Serum DBP (umol/l)	Measured Free 25OHD (pmol/l)	Calculated Free 25OHD (pmol/l)	PTH (ng/l)
Baseline	Caucasians	34.1 (12.3)	6.6 (3.0)	17.8 (7.5)	13.6 (7.8)	44.6 (14.2)
	Asians	26.3 (13.7)	4.7 (2.3)	16.7 (10.4)	11.9 (6.8)	69.8 (38.6)
*P-value < 0.05	P value	*0.04	*0.01	0.65	0.38	*0.002
Increment	Caucasians	56.7 (18.3)	0.31 (2.0)	12.2 (13.3)	24.4 (14.5)	2.2 (14.2)
	Asians	56.2 (12.6)	0.24 (2.0)	18.1 (9.4)	29.4 (20.1)	−4.7 (27.7)
*P-value < 0.05	P value	0.90	0.90	*0.05	0.29	0.24

Conclusions
Asians may well be 25OHD deficient, and the higher PTH in Asians appears to be due to low total 25OHD, rather than the free 25OHD. DBP is lower in Asians, and this is the likely reason why free 25OHD is not lower in Asians. Despite a similar response in increment of total 25OHD between the two groups, a larger increment in free 25OHD in Asians prompts us to re-consider the existing "one size fits all" approach for vitamin D treatment.

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Cancer and bone: basic, translational and clinical

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The cigarette smoking condensates effects on microRNA regulation in calcified of bladder carcinogenesis and progression

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Backgrounds
Bladder cancer is the most common malignancy and also highest mortality of the urinary tract cancer. Urothelial cell carcinoma (UCC) is the most histopathological subtype of bladder cancer. Epidemiological studies have indicated that bladder cancer risk for cigarette smoking is more than twice than never smoking and that current cigarette smoking triples bladder cancer risk relative to never smoking. Cigarette smoking after bladder cancer diagnosis decreases cancer therapeutic response and increases second cancer risk among cancer survivors. Since the urinary bladder is the organ that collects urine excreted by the kidneys before disposal by urination, the toxic metabolites of cigarette smoking may store in urinary bladder for a longer period which increases the possibility of cell transformation of urothelial cells or UCC cells and therefore increases the risk in calcified of bladder carcinogenesis or bladder cancer progression. Administration of cigarette smoking condensate (CSC) may be useful to realize overall inductive mechanisms of cigarette smoking during calcified of bladder carcinogenesis progression. Otherwise, voltage-gated Ca^{2+} channels were thought to be a main path for Ca^{2+} entry, also it became clear that a variety of other Ca^{2+}-conducting channels may extremely effects in calcified bladder function.
Materials & methods
Calcified of human UCC cell line T24 and the immortalized normal proximal tubule epithelial cell line SV-HUC-1 were employed for analyzing biological effects and molecular regulation of CSC. T24 and SV-HUC-1 are continuously exposed to 0.1, 1, 4 μg/ml (0.1% CSC), and 10 μg/ml CSC in 0.1% DMSO for more than six months. In biological effects, CSC inductive effect on cell viability was evaluated by MTT assay and non-adhersive assay. And CSC inductive effects on cell migration and invasion were evaluated using wound-healing assay, boyden chamber transwell assay, and Matrigel-coated transwell assays. In molecular regulation, CSC modified miRNA expression profile was evaluated by quantitative real-time RT-PCR. For calcium influx factor (CIF), we investigate the gene, STIM1 which could effects calcified bladder cancer cell via CSC long term treatment.
Results
This study is designed to realize the CSC effects on microRNA regulation in calcified bladder carcinogenesis and progression. We found short-term (48 h) and long-term (6 months) treatment of CSC both increased cell viability and mobility of SV-HUC-1 normal urothelial cell line and T24 calcified of UCC cell lines. Besides, CSC treatment also modified documented UCC diagnostic miRNAs in SV-HUC-1 and modified documented prognostic miRNAs in T24.
In conclusion, we found CSC indeed promoted calcified of UCC carcinogenesis and progression via modifying miRNA expression.
Key Words: bladder cancer (urothelial cell carcinoma), cigarette smoking condensate, siRNA.

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P99

DNA-methylation status affects prognostic markers of bone metastasis from breast carcinoma

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Metastasis to bone is the leading cause of death for breast carcinoma, and much focus in the biology and therapy relays on epigenetic alterations. Since DNA-methyltransferase blockade counteracts tumour growth, we utilized 5-aza-2′-deoxycytidine (dAza) to clarify whether molecular events undergoing epigenetic control were critical for bone metastatization. Here, we studied Secreted-Protein Acidic and Rich in Cysteine (SPARC), matricellular glycoprotein associated with bone-remodelling, and Endothelin1 (ET1), important for osteomimetic properties of metastatic cells, to test the hypothesis that DNA methylation in bone metastasis orchestrated a coordinate function of SPARC and ET1. In the xenograft model, prepared with 1833 clone derived from MDA-MB231-human breast carcinoma cells, we showed strong signals for SPARC and ET1. dAza administration slowed-down metastasis outgrowth, possibly consequent to SPARC and ET1 reductions at invasive front and in the bone marrow, mostly due to loss of Twist. In metastasis bulk, Snail was partly reduced by dAza, sustaining ET1-SPARC cooperativity. In 1833 cells, ET1 Induced SPARC, and both underwent post-translational control by miRNAs: ectopic miR29a reduced SPARC expression, while ET1 down-regulation occurred in the presence of endogenous-miR98 expression. Consistently, in human specimens from patients with breast carcinoma metastasizing to bone, SPARC and ET1/ET1 receptor A (ET_AR) axis were highly expressed in dysplasia and bone metastatic cells and stroma, but not in the stroma of pair-matched primary invasive ductal carcinoma. The early identification of SPARC/ET1/ET_AR in dysplastic lesions has a prognostic value to devise therapies against metastasis engraftment. We hypothesize that local production of SPARC in the hospitable bone, possibly regulated by ET1/ET_AR axis, would be important for bone niche formation and evolution. Thus, the blockade of DNA methyltransferases leading to SPARC reduction *in vivo*, might represent a promising strategy to hamper early steps of the metastatic process affecting the osteogenic niche.

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P100

The clinicopathological implication of *GNAS* in ewing sarcoma

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The objective of our study is to determine whether *GNAS* expression correlates with pathognomonic signs by analyzing mutations, methylation status, and G-protein α subunit ($G_{s\alpha}$) expression of *GNAS* (guanine nucleotide binding protein/α stimulating) gene in Ewing Sarcoma (ES).
Formalin-fixed paraffin-embedded (FFPE) tissue samples from 77 patients with primary ES were obtained in Korea, Argentina, and Brazil, and were studied via methylation chip assay and direct sequencing of the *GNAS* gene and immunohistochemical analysis of $G_{s\alpha}$. The mutation and methylation statuses of the *GNAS* gene were examined.
Immunohistochemical results were measured with respect to proportion and staining intensity. We found that *GNAS* genes in ES tumor samples were less methylated than were normal controls. No mutations were detected at exons 8 or 9 of the *GNAS* locus complex on chromosome 20q13.3, indicating that the pathogenesis of ES was not associated with *GNAS* mutation. $G_{s\alpha}$ expression correlated well with the methylation status of the *GNAS* gene. Interestingly, high $G_{s\alpha}$ expression was detected more frequently in samples from living patients than from decedents, although this was not statistically significant ($P = 0.055$).
In conclusion, *GNAS* mutation is not associated with the pathogenesis of ES tumors. This finding can be used to differentiate ES tumors from metastatic bone lesions with morphological similarity to ES tumors. Analysis of the methylation status of the *GNAS* gene and immunohistochemical $G_{s\alpha}$ expression suggests that hypermethylated *GNAS* gene (low $G_{s\alpha}$ expression) in ES may be related to unfavorable progression with a non-significant trend.

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P101

Bisphosphonates and the risk of breast cancer in osteoporotic women: a population-based study

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Background
Bisphosphonates (BP) are widely used in osteoporosis treatment. By inhibiting the mevalonate pathway, bisphosphonates may affect cell function and survival, including the viability of tumor cells. Recently, a protective effect of bisphosphonates on breast cancer risk has been suggested by several studies, which were unable to exclude the possibility of a confounder effect due to low cumulative exposure to estrogen in osteoporotic women vs controls.
Study objective
To assess the association between different levels of bisphosphonate exposure and breast cancer incidence in a cohort of osteoporotic post-menopausal women.
Study methods
This historical prospective study was conducted using the computerized databases of Maccabi Healthcare Services (MHS). Included in the study were cancer-free women aged 55–75 who started bisphosphonate therapy between 1998 and 2012. Bisphosphonate exposure was expressed in quintiles of proportion of days covered with BP during follow-up period (PDC). Cancer incidence was ascertained by the Israel National Tumor Registry.
Results
A total of 18,122 eligible MHS members were identified. 11,717 remained for analysis with 173 cases of breast cancer diagnosed during a total follow-up period of 130,252 person-years, the mean follow up time was 7.2 years. Baseline characteristics of the study population are presented. Compared to women with a PDC with bisphosphonates of 20% or lower, the hazard ratio for breast cancer were HR = 0.95 95%CI (0.55–1.62), HR = 0.74 95%CI (0.43–1.25), HR = 0.82 95%CI (0.50–1.32) and HR = 1.32 95%CI (0.86–2.02) among women with 20–40%, 40–60%, 60–80%, and 80% or higher respectively, adjusted for age, BMI, SES, smoking status, HRT use, mammograms, physician visits, and DXA scans.
Conclusions
In the present study, we did not find any significant negative association between persistence with bisphosphonates and risk of breast cancer.

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P102

Inhibition of mTOR signaling by everolimus has concurrent anti-tumor and bone-protective effects in murine osteolytic cancer models

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Clinical data suggest that the mTOR inhibitor everolimus may have bone protective effects in addition to its anti-tumor effects in women with ER+/HER2− metastatic breast cancer receiving hormone-ablative therapies. Based on these findings, we tested the hypothesis, whether everolimus exerts concurrent anti-tumor effects while protecting the skeleton in murine models. Thus, we assessed bone metabolism and anti-tumor effects in osteolytic cancer models upon exposure to everolimus. *In vitro*, everolimus concentrations of 1 nM were sufficient to significantly ($P < 0.01$) reduce the viability of various breast cancer cell lines (MCF-7, MDA-231 and MDA-Bone). Moreover, everolimus significantly reduced the number of TRAP- positive cells in both RAW 264.7 cells and murine derived osteoclasts at concentrations of ≥ 10 nM, indicating a potent anti-osteoclast effect. Consistently, the expression of markers of osteoclast activity, including TRAP and cathepsin K was also decreased. Furthermore, reduced viability and function was observed in human-derived mesenchymal stem cells (hMSC) only when exposed to higher concentrations (≥ 100 nM) of everolimus. When immunocompetent C57BL/6 mice were inoculated subcutaneously with osteotropic, murine B16-F10 melanoma cells and exposed to everolimus at a low dose of 1 mg/kg per day for 2 weeks, tumor growth was inhibited ($P < 0.01$). This was confirmed in a subcutaneous MDA-MB-231/immuno-compromised BALB/c breast cancer mouse model. In

ovariectomized C57BL/6 mice, a dose of 1 mg/kg per day for 4 weeks significantly mitigated the suppressive effect of hormone deprivation on femoral bone mass assessed by μCT ($P<0.05$). In summary, our data indicate that low concentrations of everolimus are capable of concurrently inhibiting tumor growth while preserving bone mass *in vivo*. These results warrant further research on the potential bone protective effects of everolimus in a population exposed to a high risk of osteoporosis and bone metastases, such as women with breast cancer.

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P103

Conventional and Pagetic Giant Cell Tumor of bone: distinct clinical features are defined by different genetic background and histological appearance

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Conventional Giant Cell Tumor of Bone (GCT) is an aggressive bone tumor characterized by malignant mesenchymal stromal cells, responsible for its unusually high population of multinucleated osteoclast-like giant cells. GCT could arise in bones affected by Paget's disease of bone (GCT/PDB) with a different clinical profile regarding the age-onset of the neoplasm (30 years vs 50 years) and the skeletal localization (appendicular skeleton vs cranio-facial bones), let hyphotesize a different genetic signature for these two forms of the tumor. Somatic mutations in H3F3A were described as a genetic defect of conventional GCT (92%), that we confirmed in our cohort of 44 conventional GCT cases. Recently, using whole-exome sequencing approach in a large PDB pedigree with four cases of GCT/PDB, we identified the c.2810C>G (p.Pro937Arg) missense mutation in the Zinc Finger Protein 687 gene (ZNF687), that precisely co-segregated with the clinical phenotype in all affected family members. The sequencing of seven unrelated GCT/PDB individuals identified the same mutation in all individuals, unravelling a founder effect. The absence of H3F3A mutations in these cases confirmed a different genetic background for GCT and GCT/PDB. We also identified an exquisite correlation between the mutation and the histological appearance of the tumor, with GCT/PDB showing a higher number of osteoclast-like giant cells (twice), with about 50% of nuclei per cell more than conventional GCT. Biochemically, GCT/PDB also showed a different profile as a consequence of the absence of the up-regulation of conventional GCT markers (FGFR2IIIc). In conclusion, the distinct clinical features of pagetic and conventional GCT are associated with different genetic background, resulting in a certain biochemical behaviour and histological appearance of the tumor.

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P104

Long-term effect of aromatase inhibitor on bone mineral density, trabecular bone score, and hip geometry in postmenopausal women with primary breast cancer

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Background
Aromatase inhibitors (AIs) increase the risk of fracture in patients with breast cancer. Therefore, we aimed to evaluate the long-term effects of AIs on bone health in postmenopausal women with primary breast cancer.
Patients and methods
We performed a retrospective longitudinal observational study in patients with breast cancer who were treated by AIs for ≧3 years (baseline T-score >–2.5). Patients with previous anti-osteoporosis treatment or those who were given bisphosphonate during the AI treatment were excluded from the analysis. We serially assessed bone mineral density (BMD), lumbar spine trabecular bone score (TBS), and hip geometry using dual-energy X-ray absorptiometry.
Results
Of the 321 included patients (mean age: 58.8 years), 112 patients (34.9%) underwent previous chemotherapy. The BMD significantly decreased from baseline to 5 years at the lumbar spine (–6.15%), femur neck (–7.12%), and total hip (–6.53%). Lumbar spine TBS also significantly decreased from baseline to 5 years (–2.12%) independent of lumbar spine BMD. The annual loss of lumbar spine BMD and TBS slowed down after 3 years and 1 year of treatment, respectively, although there was a relatively constant loss of BMD at the femur neck and total hip up to 4 years. Cross-sectional area, cross-sectional moment of inertia, minimal neck width, femur strength index, and section modulus significantly decreased, although the bucking ratio increased over the treatment period (all $P<0.001$); these changes were independent of total hip BMD. No significant differences were observed in time-group interactions for BMD, TBS, and hip geometry according to the presence of previous chemotherapy.
Conclusion
Long-term adjuvant AI treatment negatively influenced BMD and bone quality in patients with breast cancer. This study suggests that a monitoring and preventive strategy could be beneficial for patients with breast cancer who are starting or receiving AI treatment.

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P105

Effects of the female hormone inhibin-A in vivo: potential contribution to the antitumour effect of Zoledronic acid

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Background
Breast cancer clinical trials have shown an enhanced anti-tumour activity of bone-targeted agents in postmenopausal patients. We have reported that zoledronic acid (ZOL) decreases serum levels of the tumour promoter follistatin in postmenopausal women and also inhibits expression of follistatin by breast tumour cells both *in vitro* and *in vivo*. We hypothesised that inhibin-A (InA) and ZOL may be altering bone levels of follistatin and its bound tumour suppressor protein activin, to enhance the anti-tumour effects of ZOL in postmenopausal women.
Objectives
To evaluate the effects of InA+ZOL on the bone microenvironment, including the levels of follistatin/activin as well as breast tumour cell homing, using an *in vivo* model of post-menopausal bone.
Methods
Twelve-week old BALB/c nude mice were ovariectomised (OVX)/sham-OVX and implanted with sub-cutaneous osmotic pumps delivering 10/60/120 ng/day InA/saline for 4 weeks. ZOL (100 μg/kg, IP) was injected weekly. DiD-labeled MDA-MB-231 cells were injected IC after 4 weeks. Serum was processed to ELISA for InA, TRAP and P1NP. Calvaria were crushed and supernatant processed to ELISA for activin and follistatin. Hind legs were fixed in 4%PFA and analysed using μCT+TRAP stain or frozen and processed for detection of DiD-labelled tumour cells by 2-photon microscopy.
Results
One hundred and twenty ng/day InA increased serum levels (mean InA (pg/ml); OVX-control=29.1, OVX-InA=68.3 $P=0.0079$) and prevented OVX-induced bone loss (mean BV/TV (%); OVX-control=4.4, OVX-InA=7.8 $P=0.0079$). InA did not affect numbers of Ob/Oc but, in OVX animals, increased TRAP (mean TRAP (U/I); OVX-control=2.3, OVX-InA 2.7, $P=0.008$) and decreased calvaria activin (mean activin (pg/ml); OVX-control=65.2, OVX-InA=37.2, $P=0.01$) but did not affect the number of DiD positive events/mm^3. ZOL decreased serum follisatin in OVX animals (mean follistatin (pg/ml); OVX-control=67.8, OVX-ZOL=48, $P=0.036$).
Conclusions
In OVX animals, InA alters bone activin levels but does not affect tumour homing to bone. ZOL decreases bone follistatin levels *in vivo*, which may explain the serum effects seen in postmenopausal women after ZOL treatment. Combined effects of InA and ZOL on tumour growth in bone require investigation.

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P106

The Rho GTPases RhoA and CDC42 mediate apoptosis by a combination of statins and zoledronic acid in human bone-seeking breast cancer cells

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Breast cancer is the most frequent malignancy in women and frequently results in osteolytic bone metastases. Amino-bisphosphonates are a standard bone protective therapy and, similarly to statins, inhibit the mevalonate pathway that is crucial for posttranslational protein modifications (farnesylation and geranylation). Direct anti-tumor effects of amino-bisphosphonates and statins have been suggested but high concentrations are necessary to achieve meaningful effects. Our study aimed to assess the potential of combining amino-bisphosphonates with statins to reach anti-tumor effects at lower doses.
We demonstrate that the expression levels of both targets of statins and amino-bisphosphonates, the 3-hydroxy-methyl-glutaryl-CoA reductase (HMGCR) and the farnesyl diphosphate synthase (FDPS), show a positive correlation in clinical samples of breast cancer ($P \leq 0.0001$) pointing to a potential benefit of simultaneously targeting both enzymes. Treating different osteotropic human cancer cell lines (MDA-MB-231, MDA-BONE, MDA-MET, MDA-453s, and PC3) with a combination of low concentrated statins (atorvastatin, simvastatin and rosuvastatin) and zoledronic acid resulted in significant anti-tumor-effects (50% reduction of cell vitality and fourfold induction of apoptosis; $P < 0.05$). Apoptosis was the result of blocked geranylation rather than farnesylation. Rho GTPases like RhoA, Rac1, and CDC42 represent a major class of geranylated proteins. Surprisingly, the treatments with mevalonate pathway inhibitors, individually or in combination, caused an accumulation of GTP-bound RhoA and CDC42 but not of Rac1. Importantly the knockdown of RhoA and CDC42 prior to the treatment with simvastatin and zoledronic acid reduced the caspase 3/7 activation by up to 60% and the cleaved PARP accumulation by up to 80% in MDA-MB-231 human breast cancer cells ($P < 0.01$). The observations point to a contribution of these Rho GTPases in the anti-tumor effects by statins and zoledronic acid.
Our results suggest the combination of statins and zoledronic acid as an effective approach for the adjuvant treatment of breast cancer.

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P107

Biological effects of Cabozantinib on bone microenvironment

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Background
Cabozantinib (CBZ) is a receptor tyrosine kinase inhibitor with activity against MET, VEGFR2, FLT3, c-KIT, and RET. Pre-clinical studies in models of prostate cancer bone metastasis demonstrated that CBZ treatment induced both a suppression of tumour growth and an alteration in bone remodelling, suggesting that both tumour and bone microenvironment represented potential CBZ targets. This is the first study exploring the potential direct activity of CBZ in bone using a totally human model of primary osteoclasts (OCLs) and osteoblasts (OBLs).
Methods
Primary OCLs were differentiated from CD14+ monocytes; primary OBLs 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. Gene expression analyses was performed by Real Time PCR and OPG and RANKL protein secretion measured by ELISA.
Results
Our results showed that non-cytotoxic doses of CBZ had a statistically significant inhibitory effect on OCL differentiation ($P = 0.0145$) and bone resorption activity ($P = 0.0252$). Moreover, we found that CBZ down-modulated the expression of OCL marker genes: TRAP ($P = 0.006$) and CATHEPSIN K ($P = 0.004$). On the other hand CBZ treatment had no a significant impact on OBLs vitality, differentiation and activity. Intriguingly we found that CBZ induced in OBLs an alteration of RANKL/OPG balance increasing OPG mRNA levels ($P = 0.015$) and down-modulating RANKL expression ($P < 0.001$). A significant drop in RANKL secretion ($P = 0.043$) and an increase of OPG production ($P = 0.004$) was confirmed by ELISA.
Conclusion
Overall, this is the first evidence in human of the 'direct' effect of CBZ on OCLs and 'indirect' osteoblast-mediated through a modulation of RANKL/OPG balance. These multiple effects of CBZ on the cells of bone microenvironment are consistent with its effectiveness in reducing lesions from prostate cancer metastases.

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P108

Clinical and experimental evidence suggest a protective effect of Paget's disease of bone against skeletal metastasization from solid tumors

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Paget's disease of bone (PDB) is a common disorder of bone metabolism characterized by focal areas of excessive and rapid bone resorption and formation, leading to bone pain, deformity and fractures. Despite the well documented increase in the risk of primary bone tumors due to neoplastic degeneration of pagetic tissue, a large retrospective analysis suggested that patients with prostate cancer and PDB have delayed time to bone metastases and improved overall survival than do patients with prostate cancer alone (*Br J Cancer* 2012;107:646–51). This association is unexpected since metastatic cells seed in skeletal sites under active turnover containing dense marrow cellularity and high bone turnover markers (as typically observed in PDB) have been consistently related with negative clinical outcomes and increased skeletal metastasization from prostate cancer or other solid tumors. Based on this observation, we performed a survey of retrospective clinical databases in 893 patients from the Italian PDB Registry (of whom 79 with documented prostate or breast cancer) and we observed a significantly decreased prevalence of skeletal metastases from both tumors with respect to the estimates from the general population, with a total absence of metastases in the 43 cases with the occurrence of cancer after the diagnosis of PDB, despite a mean observation period of 10.5 ± 4.3 years. Consistent with this observation, in a preliminary *in vitro* analysis, the conditioned media (CM) from osteoclasts derived from peripheral blood leucocytes of patients with active PDB was able to decrease the proliferation of the osteotropic breast cancer cell line MDA-MB-231, as assessed by the MTT test, with respect to CM of controls. Taken together, these results indicate that PDB patients have a distinct bone condition or a unique bone microenvironment that protects them from bone metastasis and strongly encourage further analyses in order to identify the underlying molecular mechanisms.

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P109

Antiproliferative properties of oleuropein in human osteosarcoma cells

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Background
Cancer is one of the leading causes of death worldwide. Natural products have been regarded as important sources of potential chemotherapeutic agents. In this study, we evaluated the antiproliferative activity of oleuropein, an olive oil compound traditionally found in the Mediterranean diet.
Design and Methods
The antiproliferative activity on two human osteosarcoma cell lines (MG-63 and Saos2) was evaluated *in vitro* using the MTT colorimetric methods. The median inhibitory concentration values (IC_{50}) and 95% confidence interval (CI) for oleuropein were established by the weighted Probit method for both cell lines at 24, 48 and 72 h of exposure.

Results
Oleuropein exhibited obvious cytotoxic effects on human osteosarcoma cells in a concentration- and time-dependent manner. Statistical analysis of IC_{50} by the Probit regression method suggested that oleuropein had similar toxic effects on both cell lines tested (IC_{50} range from 247.41 to 474.97 μM for MG63 cells and IC_{50} range from 798.69 to 359.91 μM for Saos2 cells).
Conclusion
This is the first study showing an antiproliferative activity of oleuropein in human osteosarcoma cell lines.

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P110

Role of the receptors FZD8 and RYK in mediating the anti-tumor 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 prostate cancer (PCa). We previously showed 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 determine the role of two Wnt receptors (FZD8, RYK) and their influence on the WNT5A-induced effects on PCa cells.
The expression of the Wnt receptors Frizzled (FZD) 8 and RYK was analyzed in three human (PC3, C42B, MDA-PCa-2b) and two mouse (RM1, TRAMP-C2) PCa cell lines. RYK (CT-value 18-23) is higher expressed in all prostate cancer cell lines than FZD8 (CT-value 24-33). Further, FZD8 showed the lowest expression levels in the osteoblastic PCa cell line MDA-PCa-2b (CT-value 33). To determine which receptor mediates the pro-apoptotic and anti-proliferative effects of WNT5A in PCa, we knocked down RYK and FZD8 with specific siRNA in PC3 cells 24 h before the induction of WNT5A overexpression. Knock-down of FZD8 induced a further increase of apoptosis after WNT5A overexpression (2.2-fold, $P<0.01$). In contrast, knock-down of RYK fully reversed the induction of apoptosis after WNT5A overexpression ($P<0.05$). Interestingly, the decrease of proliferation after WNT5A overexpression was not reversed by neither knock-down of RYK nor FZD8, suggesting another receptor involved in this process. After knock-down of RYK, WNT5A was still able to suppress proliferation by 28%. Of note, FZD8 knock-down even further decreased (from 27 to 55%) proliferation after WNT5A overexpression. To validate our findings *in vivo*, a cDNA array containing samples from nine healthy and 39 patients with prostate cancer was evaluated for WNT5A and RYK expression. RYK mRNA expression correlated highly positively with WNT5A expression ($r^2=0.9309$, $P<0.001$).
These data suggest that RYK, but not FZD8, mediates the pro-apoptotic effects of WNT5A on prostate cancer cells.

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P111

Changes in monocyte and NK-like cell subpopulations in the peripheral blood of patients treated with zolendronic acid

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Osteonecrosis of the jaws (ONJ) is a relatively new adverse effect associated with bisphosphonate therapy, but no causal association has been established. By definition, a patient is considered to suffer from ONJ if he has current or previous treatment with a bisphosphonate, exposed bone in the maxillofacial region that has persisted for more than 8 weeks and no history of radiation therapy to the jaws. It has been proposed that ONJ could be linked with impaired topical immune response due to the toxicity exerted by bisphosphonates on macrophages, further to their toxicity in osteoclasts. The aim of the present study was to test this theory and examine the effect of zolendronate administration in peripheral blood monocyte and NK-like cell populations.
Methods
In this pilot study, we included six breast cancer patients, on hormone therapy, who were treated with zolendronic acid for a period of at least 6 months and who did not receive chemotherapy for a period of at least 1 year. Monocytes (CD45+, CD14+CD23+, CD14+CD23−, CD14−CD23+, CD14+CD123+, CD14+CD123− and CD14−CD123+) and NK-like cells populations (CD45+CD3+, CD45+CD3-, CD45+CD16+CD56+, CD3+CD16+CD56+, CD3−CD16+CD56+, CD3+CD16+CD56− and CD19+CD45+) were examined (immune phenotype quantified sampling profile – IPQSP via flow cytometry and antibody-based fluorescence). The IPQSP was conducted prior to zolendronic acid infusion and 48 h after, on peripheral blood samples.
Results
In this preliminary clinical sample, we were able to detect a significant increase in CD14−CD123+ (Pearson's χ^2, $P=0.042$), CD45+CD3− ($P=0.004$), CD3+CD16+CD56+ ($P=0.036$) and CD3-CD16+CD56+ ($P=0.026$) populations. We were also able to detect a significant decrease in the CD45+CD16+CD56+ population ($P<0.0001$).
Conclusions
We demonstrate changes in the monocyte and NK-like cell populations, following zolendronic acid administration in patients with breast cancer on hormone therapy. The effects of bisphosphonates in those cells may be causatively linked with the development of ONJ.

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P112

Myeloid-derived suppressor cells (MDSC) mediate the positive feedback loop of prostate tumor-bone interactions

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Advanced-stage prostate and breast cancer patients commonly develop bone metastases, accounting for significant clinical problems such as pain, fracture, immobility and death. Bone is comprised of diverse cell types that are potentially involved in metastatic progression. However, how cancer cells interact with these cells within the bone microenvironment to support their expansion and activity remains unclear. Recent data from our laboratory highlighted a novel feed-forward mechanism underlying the interactions between prostate cancer cells and a pro-tumorigenic subset of immature myeloid cells in the bone marrow. Briefly, levels of tumor derived parathyroid hormone-related protein (PTHrP) correlated with myeloid-derived suppressor cell (MDSC) recruitment in the tumor tissue, with further evidence for tumor-derived PTHrP potentiates MDSC activity and function within the bone marrow of tumor hosts. Mechanistic investigations *in vivo* revealed that PTHrP elevated Y418 phosphorylation levels in Src family kinases in MDSC via osteoblast-derived interleukin-6 and VEGF-A, thereby upregulating MMP-9. Taken together, our results showed that prostate cancer–derived PTHrP acts in the bone marrow to potentiate MDSCs, which are recruited to tumor tissue where they contribute to tumor angiogenesis and growth. Furthermore, we demonstrated that osteal macrophages mediate anabolic actions of parathyroid hormone. Collectively, this presentation will describe the distinct roles of myeloid-lineage bone marrow cells in bone during the progression of bone metastasis, and will discuss the potential therapeutic approaches.

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P113

Dendritic glycopolymers as efficient drug delivery systems for retarded release of bortezomib from calcium phosphate cements

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Calcium phosphate cements (CPC) are used as bone graft substitute, e.g. in the treatment of lytic bone lesions in multiple myeloma. CPC provide crucial advantages, such as osteoconductivity, biodegradability and the potential drug loading. Though, it lacks retarded drug release for short-/long-term treatment due

to the free diffusion of small molecules through the micropores in the CPC.
Thus we present dendritic glycopolymers (DG) consisting of poly(ethylene imine) (PEI) decorated with oligo(glutamic acid) (PGlu) and/or maltose and maltotriose (Mal; Mal-III), respectively, as nanocarriers for the proteasome inhibitor bortezomib (BZM) in CPC. Thus, in aqueous solution the drug delivery systems exhibit a sufficiently high drug uptake of 54% for PEI-Mal and 73% for PEI-Mal-III, but only 35% for PEI-PGlu-Mal. Furthermore from DG/CPC composite a significant retarded BZM release is determinable. This has been observed with different polymer/drug ratios. In the table BZM release values at 37 °C are shown for 1 g CPC containing 50 µg BZM and 100 µg GD. PEI-PGlu-Mal provide the most suitable release profile for BZM.
The mechanical and morphological properties of the bone substitute are not influenced by the DG. The compressive strength remains at 27–29 MPa for the CPC with and without different GD-concentrations. Moreover biocompatibility of the GD was tested by lactate dehydrogenase and alkaline phosphatase activity. GD do not affect proliferation and differentiation of hMSC: e.g. the cell number after 14 days of treatment with PEI-PGlu-Mal solutions retains constant between 12,000 and 10,000. Concluding the results CPCs loaded with BZM complexed by GD are promising materials for bone reconstruction in terms of short-/long-term treatment of cancer damaged bones.

Time	BZM without GD	PEI-Mal/BZM	PEI-Mal-III/BZM	PEI-PGlu-Mal/BZM
3 h	28.0%	19.3%	16.0%	10.5%
8 h	47.2%	37.5%	30.1%	15.2%
24 h	66.4%	54.9%	51.4%	34.9%
96 h	70.4%	61.1%	63.3%	54.2%

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P114

Increased zinc accumulation in mineralized osteosarcoma tissue

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Abnormal tissue levels of certain trace elements such as Zinc (Zn) were reported in various types of cancer. Little is known about the role of Zn in osteosarcoma. Using confocal synchrotron radiation micro X-ray fluorescence analysis, we characterized the spatial distribution of Zn in high-grade (G3) sclerosing osteosarcoma of nine patients (4 f/5 m; 7 knee/1 humerus/1 femur) following chemotherapy and wide surgical resection. The study was done in accordance with the Helsinki Declaration.
Zn levels in mineralized osteosarcoma tissue were compared to levels in adjacent normal tissue. Quantitative backscattered electron imaging (qBEI) as well as histological examinations were also performed. On average, the ratio of medians of Zn count rates (normalized to calcium) in mineralized tumor tissue was about 6 times higher than in normal tissue. There was no difference in Zn levels between tumor fraction areas with a low and a high fraction of mineralized tissue, which were clearly depicted using qBEI. Moreover, we found no correlation between the Zn values and the type of tumor regression according to the Salzer-Kuntschik grading. The underlying mechanism of Zn accumulation remains unclear. Given the emerging data on the role of trace elements in other types of cancer, our novel results warrant further studies on the role of trace elements in bone cancer.

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P115

Polycystin-1 is involved in osteosarcoma pathobiology

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Osteosarcoma is the most common primary bone-derived tumor. However, the pathogenic molecular mechanism(s) underpinning osteosarcoma development and metastasis remain elusive. The polycystins PC1 (polycystin-1; encoded by PKD1) and PC2 (polycystin-2) are central players in mechanotransduction, a process that can influence all steps of the invasion/metastasis cascade. Recent studies from our laboratory provided, for the first time, a direct link between mechanosensing polycystins and colorectal cancer.
Our aim was to investigate the potential role of PC1 in osteosarcoma pathogenesis.
Immunohistochemical expression of PC1 was evaluated in human paraffin-embedded osteosarcoma tissues. MG-63 osteosarcoma cell line was cultured *in vitro* and functional assays were performed following PC1 extracellular inhibition in order to assess cell proliferation and migration. PKD1 knockdown was achieved in MG-63 cells transfected with PKD1-specific siRNA.
PC1 presented basic levels of endogenous protein expression and predominant nuclear localization in osteosarcoma tissues. Functional inhibition of PC1 was associated with increased cell proliferation and migration in MG-63 cells. PKD1 knockdown was accompanied by activation of key components of the Wnt and PI3K/Akt/mTOR signaling pathways.
PC1 is implicated in the molecular mechanism(s) underlying osteosarcoma pathobiology.

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P116

Signaling network of mirnas regulating bone metastasis in lung cancer

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We found that miR-335 is reduced in SBC-5, a human lung cancer (LC) derived cell lines inducing osteoclast formation by expressing RANKL; reviving miR-335 expression in SBC-5 results in decrease of its proliferation, invasion, migration and clone formation *in vitro*; reviving miR-335 expression in LC model leads to reduction of bone metastasis (BM) *in vivo*. It was confirmed that miR-335, inhibiting the formation and activity of osteoclasts and reducing the proliferation of tumor cells by targeting RANKL and insulin-like growth factor-I receptor (IGF-IR) expression, is an important miRNA regulating bone metastasis of lung cancer (LCBM).
MiR-21 level in SBC-5 and its supernatant were found significantly increased, while expression of miR-21 was elevated in serum of LCBM patients. Providing inhibitor of miR-21 attenuates SBC-5 induced osteoclast formation. It suggests high expression of miR-21 in LC may be released into blood circulation and is closely related to BM.
Our data shows changes of miRNA profile (miR-335,miR-21, etc.) occur in some LC cells according to the environment and genetic factors. Abnormally expressed miRNAs could not only be released into blood circulation due to necrosis or secretion of LC cells but also result in abnormal expression of BM related proteins, which make LC cells acquire the characteristic of BM. Arriving bones via blood circulation, those free miRNAs and bone-seeking LC cells interact with osteoclasts/osteoblasts in bone microenvironment and result in osteolytic lesion by destroying balance between bone formation and resorption. Growth factors released from destroyed bone promote growth of LC cells in bone, which cause the osteolytic BM finally. Including miRNAs and their upstream/downstream proteins, the signaling network regulates occurrence and development of LCBM, which may serve as biomarker of diagnosis and target of therapy for LCBM.

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P117

Contribution of multiple myeloma-derived exosomes to bone disease

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Bone disease is the most frequent complication in multiple myeloma (MM) resulting in pain, bone fractures, spinal cord compression and hypercalcemia. Within the bone marrow microenvironment (BMM), MM cells interact with bone cells to enhance bone resorption activity and compromise new bone formation mechanism; in turn, BMM provides a survival and drug resistance framework by interaction of MM cells with bone marrow components. Exosomes are important mediators of crosstalk between MM cells and BMM, as new evidence indicates that exosomes secreted by MM cells positively modulate osteoclast differentiation, playing a key role in destructive osteolytic processes. Here, we proposed that MM cell-derived exosomes contribute to Osteoblast/Osteoclast uncoupling in osteolytic lesions, playing a critical role in the regulation of RANK/RANK-L pathway inside the BMM. Moreover, we aimed to investigate effect of osteoclasts differentiated after treatment with MM-cell derived exosomes (OCs MM-exo treated) on survival and apoptosis of MM cells, by better understanding the intricate networks among molecules which control these processes.
MM cell lines cultured with a conditioned medium obtained by OCs MM-exo treated, showed increased expression of pro-survival and anti-apoptotic factors; of note, MM cells produced much more RANK-L and DKK1 factors, critical regulators of bone remodelling processes, compared to untreated cells. Proteomic analysis of exosomes isolated from MM cells revealed the presence of extracellular matrix proteins, as well as factors correlated with bone remodelling mechanisms. Luminex multiplex cytokine analysis highlighted presence of proteins of uPA system, involved in important biological events such as cancer cell invasion, adhesion and bone matrix degradation. In conclusion, our results show that MM-exosomes affect cancer cell survival, and anti-apoptosis mechanisms, sustaining therefore MM bone disease. Finally, further studies on the molecular content of exosomes will greatly improve the understanding of the nanovesicles role on the onset and severity of osteolytic lesions in MM.

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P118

Menin is a tumor suppressor in bone – a novel benign jaw tumor mouse model

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Benign jaw tumors including ossifying fibroma (OF) can be often incidentally diagnosed due to their slow-growing and painless characteristics. In cases of massive growing jaw tumors, it can result in deformity of teeth, infection and intracranial complications. Depending on the location of tumor lesion, it may not completely resected and recur after the primary operation, which may require subsequent operations.
Molecular mechanisms and the etiology of those bone tumors are scarce and their eludication will allow to developing new alternative therapies to reduce complicated facial surgery.
Here we established the tumor suppressor gene *Men1* (multiple endocrine neoplasia type1) as a novel gate keeper for the development of OF. We found that *Men1* deletion in mesenchymal stromal cells/osteoblast progenitors caused benign jaw tumor OF in mice with 100% incidence. This tumor lesion was characterized by less bone mass along with high bone resorption, and expanded stroma. Consistently, mesenchymal stromal cells isolated from OF (OFMSCs) were not able to perform mineralization, explaining low bone mass in the tumor lesion. OFMSCs are excessively proliferative which was reduced by *Men1* overexpression, showing a slower transition from G0/G1 to S-phase in *Men1*-overexpressed OFMSCs.
We found TGF-β target gene expression was upregulated in tumor-bearing mandibles and OFMSCs lacking *Men1* displayed an increased sensitivity towards TGF-β treatment. Finally, we discovered that *Men1*-deficient OFMSCs down regulated the cyclin dependent kinase (CDK) inhibitor p21, explaining increased proliferation.
Our findings show for the first time that osteoblast lineage specific *Men1* deletion causes jaw tumors in mice by inhibiting differentiation of osteoblast progenitor cells and by promoting proliferation, presumably through affecting TGF-β signaling and p21 expression. We developed here a new mouse model of rare ossifying fibromas in the jaw that can be exploitet to develop novel treatment strategies.

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P119

Abstract unavailable.

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P120

The role of acidic microenvironment in the context of osteolytic carcinomas

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The microenvironment of osteolytic metastases includes carcinoma cells derived from the primary lesion as well as bone-forming and bone-resorbing cells, namely osteoblasts (OB) and osteoclasts (OC). At this site, both the high glycolysis of cancer cells, and the bone resorption process result in a very acidic milieu. This, in turn, induces the surrounding stroma and OB to secrete pro-inflammatory cytokines and growth factors that promote tumorigenesis and cancer-associated osteolysis via OC activation. Here, we investigated the role of acidic microenvironment in osteolytic carcinomas and its effect on the interplay between cancer and stromal cells.
The glycolytic rate and capacity of carcinoma cells were determined by Seahorse Extracellular Flux (XF-96) analyzer. The level of expression of the main pumps and transporters involved in the regulation of cell metabolism and acidification, under both normoxic and hypoxic conditions, were determined by Real-time PCR. To evaluate the effect of tumor acidification on the stroma of bone metastases, mRNA expression of IL6, IL8, RANKL, and M-CSF was assayed by Real-time PCR in OB exposed to acidic medium. Additionally, to evaluate the effect of the secretome of tumor-associated stroma on OC differentiation and activity, human OC derived from peripheral blood mononuclear cell (PBMC) were cultured with the supernatant of OB pre-conditioned in acidic medium.
Our results confirmed a high glycolitic and acidification activity in carcinoma cell lines, an increased gene expression of the proton pump Vacuolar ATPase (V-ATPase), of the carbonic anhydrase CA-9, and of the GLUT-1 transporter, both in mammary and in renal carcinoma cells. Furthermore, acidity induced an increase of IL6 and IL8 expression in OB that in turn promoted OC formation. Our results confirm that, in bone metastases, extracellular acidification by tumor cells promote the release of pro-mitogenic and pro-osteoclastogenic factors from the surrounding stroma.

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P121

MCT1 as a novel target for the treatment of osteolytic bone metastases

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Bone metastasis (BM) is a dismal complication of cancer, occurring frequently in patients with advanced breast carcinoma. During metastatic progression, carcinoma cells harness osteoclast (OC) activity, promoting osteolysis. To adapt to hypoxia and/or to support proliferation, carcinoma cells adopt primarily glycolysis for energy production, therefore releasing lactic acid in the microenvironment through monocarboxylate transporter 4 (MCT4). Stressed by tumor cells, osteoblasts (OB) can also switch to a more glycolytic metabolism, further promoting lactate release. Here, we hypothesized that extracellular lactate is uptaken by OC through MCT1 to fuel oxydative phosphorylation (OXPHOS), thereby promoting the osteolytic process. We used human primary cultures of OC and OB, wt breast carcinoma MDA-MB-231 (wtMDA-BM-231) cells and MDA-MB-231 clone prone to form bone metastasis (bmMDA-MB-231). OC differentiation and activity were analyzed by TRAP/nuclei staining, and osteolyse assay. MCTs expression was evaluated by RT-qPCR and western Blot. Metabolic analyses were performed by Seahorse XF96 and CMA600 analyzers, JC-1 staining and ATP assay. We found an increased expression of MCT1 in OC during the differentiation process, a dose-dependent uptake of ^{14}C-lactate by OC and that OC rely on OXPHOS as the main source for energy production, whereas monocytic precursors have a higher glycolytic rate. Finally, exposure of OC to sodium lactate promoted OXPHOS. As a confirmation of our hypothesis, OB, wtMDA-MB-231 and bmMDA-MB-231 express high levels of MCT4 and do not express MCT1. Our data demonstrate that lactate released by tumor cells and OB into the BM microenvironment can be uptaken by OC through MCT1 to promote the BM osteolytic process. MCT1 is therefore a promising target for the treatment of BM.

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P122

Acetate metabolism in Multiple Myeloma identifies ^{11}C-Acetate PET as a novel strategy to image bone disease and response to treatment in preclinical models

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Multiple Myeloma (MM) is a malignancy of Plasma Cells (PC), characterized by severe osteolytic lesions but poor ^{99}Tc-MDP uptake in bone scans due to osteoblast inhibition. We hypothesized that high demands for membrane biosynthesis in tumour PC would enhance monocarboxylic acid anabolism and uptake, which could be exploited for treatment and molecular imaging. Here, we tested the efficacy of clinically available ^{11}C-Acetate PET to detect myeloma and quantitatively image treatment response in vivo, and characterized acetate metabolism in myeloma cells (MMC).

Experimental design

^{11}C-Acetate PET/CT imaging and bio-distribution were performed in subcutaneous (5TGM1, U266 and OPM2) and orthotopic (KaLwRij) MM mouse models. Tumour burden was quantified by flow cytometry, optical imaging, and serum electrophoresis (SPEP). Mice with established MM were treated with bortezomib to evaluate response with PET. In order to study acetate metabolism, we performed metabolite fate tracking with ^{13}C-edited ^{1}H NMR and challenged cells with chemical inhibitors of transport (CHC) or anabolism (orlistat) to assess single-agent or combined antimyeloma effects.

Results

^{11}C-Acetate uptake was enhanced in subcutaneous MMC tumours. Tumour-bearing leg bones showed higher ^{11}C-Acetate uptake than unaffected bones or muscle. Post treatment, ^{11}C-Acetate uptake was significantly decreased, demonstrating response. NMR showed metabolism of acetate by MMC, including incorporation into membrane lipids. Inhibition of acetate metabolism had limited effects on normal B cells and bone resident cells (osteoblasts, osteoclasts, BMSC), but induced cell death in MMC.

Conclusions

In vitro uptake of acetate from the extracellular environment is enhanced in MMC to sustain growth and viability. ^{11}C-Acetate PET detected the presence of myeloma cells *in vivo* in MM mouse models, including intramedullary disease. ^{11}C-Acetate-PET rapidly detected response to therapy *in vivo*. Our data suggest that ^{11}C-Acetate PET might be a promising tool for imaging in MM, and anabolic metabolism of monocarboxylic acids could represent a novel therapeutic target.

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P123

The SRC kinase inhibitor saracatinib limits the development of osteolytic bone disease in multiple myeloma

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Destructive bone lesions due to osteolytic bone disease (OBD) are a major cause of morbidity and mortality in multiple myeloma (MM) patients and the development of new therapeutic strategies is of great interest. In this study, we assessed the effect of SRC inhibition with saracatinib (AZD0530, AstraZeneca) on the development of MM and its associated OBD. We first determined SRC family kinase expression in the MM microenvironment and found that myeloma cells express SRC at low levels, which do not correlate with disease stage. SRC expression was found to increase during osteoclast differentiation and decrease during osteoblast differentiation. Next, we validated an inhibitory role of saracatinib on osteoclast differentiation and function. Saracatinib inhibited the differentiation and polarization of RAW264.7 and primary osteoclasts, reflected by a decrease of CTSK and DC-STAMP levels and a defective actin ring formation. This culminated in an inhibition of bone matrix resorption. In addition, saracatinib inhibited collagen secretion by MC3T3-E1 osteoblasts. In both the 5TGM.1 and 5T2MM murine myeloma models, bone destruction was markedly reduced following treatment with saracatinib, reflected by a restoration of multiple trabecular bone parameters to levels observed in healthy control mice, including BV/TV, Tb.N. and Tb.Th. These findings were corroborated by histomorphometric analyses. Although BM plasmocytosis was not affected in these mice, *in vitro* studies suggest synergism between bortezomib and saracatinib. In conclusion, we report a potent inhibitory preclinical effect of the SRC inhibitor saracatinib on the development of OBD in MM, further establishing SRC as a promising therapeutic target.

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P124

Gene expression of P2 receptors in mitotically quiescent, prostate cancer bone metastasis initiating cells

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Rationale and hypothesis

In ~70% of advanced prostate cancer (PCa) patients, incurable bone metastases are a significant cause of morbidity and mortality. It has been shown that in models of PCa, bone metastases are initiated by a minor subset ($<1\%$ of total cell population) that are mitotically quiescent and which have undergone epithelial to mesenchymal transition (EMT). Recent studies have shown that various P2 receptors regulate invasiveness/EMT in different types of cancers including PCa. Therefore, we hypothesised that alterations in the expression of P2 receptors are associated with the PCa metastasis-initiating cells.

Objectives

To study the gene expression profile of the fourteen P2 receptors in PCa metastasis-initiating cells.

Methodology

In our previous studies, we have developed methods to track quiescent tumour cells both *in vitro* and *in vivo*, based on their fluorescent lipophilic dyes retention when not dividing and shown that these cells have an increased capacity to form metastases. Using these models and quantitative RT-PCR and RNA-Seq techniques, we compared gene expression profile of P2 receptors between FACS sorted quiescent and non-quiescent populations in two human PCa cell lines, covering both osteolytic (PC3 cells) and osteosclerotic (C4 2B4 cells) bone metastases.

Results

Results showed that P2X3, X4, X5, X7, Y_1, Y_4, Y_{13}, and Y_{14} receptors transcripts were expressed in both cell lines *in vitro*. The expression of *P2RX4* and *P2RX7* were significantly increased in quiescent, metastasis initiating populations. Analysis of the expression of these genes also suggested up-regulation of *P2RX4* and *P2RX7* in PCa cells growing in bone of immunocompromised mouse as xenografts, compared to cultured populations.

Conclusion

These data suggest the involvement of P2 receptors, particularly the receptors of ATP – P2X4 and P2X7 receptor, in the development of PCa bone metastasis and

identify these genes and the pathways they regulate as potential novel therapeutic targets for preventing/suppressing metastases.

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P125

Host-derived MMP-13 promotes Multiple Myeloma skeletal colonization

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A hallmark of Multiple Myeloma is skeletal colonization resulting in painful osteolytic lesions. Matrix metalloproteinase-13 (MMP-13) is a type-1 collagenase largely expressed by mesenchymal stromal cell (MSCs) in the bone tissue. In agreement, immunohistochemical staining of human myeloma biopsies demonstrated the localization of MMP-13 to the stromal compartment with expression in a subset of myeloma cells. This observation was further supported by studies showing that co-culture of myeloma cells with human MSCs, strongly up regulated MMP-13 expression in MSCs (1.81 LogFC, $P<0.05$.) Given the role of MMP-13 in bone matrix turnover and the osteolytic nature of the disease, we hypothesized that host-derived MMP-13 plays a potentially key role in myeloma progression and colonization of the skeleton.
Inoculation of 5TGM1-Luc into immunodeficient wild-type or MMP-13$^{-/-}$ mice revealed that MMP-13 significantly contributed to overall survival (mean 39 vs 43 days; $P<0.05$). Unexpectedly, we observed no difference in tumor burden between the groups. However, MMP-13$^{-/-}$ mice had significantly more bone volume compared to controls as determined by histomorphometry, X-ray and μCT (0.5 vs 0.23 ratio; $P<0.05$). *In vitro,* we noted that fewer and smaller osteoclasts formed in the MMP-13$^{-/-}$ bone marrow cultures compared to control (Mean number 89 vs 16; $P<0.05$). This was associated with a reduced bone resorptive ability when osteoclasts were culture on a bone mimetic (24% reduction; $P<0.05$). In keeping with immunohistochemical studies and the literature, immunofluorescent staining of wt bone marrow revealed MMP-13 expression to be absent from osteoclasts and largely confined to MSCs. These data imply that MMP-13, derived from bone mesenchymal stromal cells contributes to myeloma progression by enhancing osteoclastogenesis and bone degradation. The tissue restrictive expression of MMP-13 makes it an attractive therapeutic target for the treatment of multiple myeloma.

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P126

Interactions between cancer and osteocytes in bone

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Cross talk between tumour cells and the host microenvironment plays a critical role in tumour progression. There have been multiple studies that have highlighted the importance of proteins secreted by tumour cells that then act on the tumour microenvironment and by proteins secreted by the host microenvironment that promote tumour progression at the metastatic niche. However, little is known regarding the role of physical forces on tumour progression.
Bone has several unique properties including calcified matrix and specialized cells including osteoclasts, osteoblasts and osteocytes. While the role of osteoclasts and osteoblasts have been described in the pathophysiology of bone metastases, there is a dearth of knowledge regarding the role of osteocytes in bone metastasis. Osteocytes are the most prevalent cell present in mineralized bone and are considered the main mechanotransducing cells. Thus it is plausible that osteocytes can respond to tumour-generated physical forces, which in turn induce signalling in the osteocytes resulting in their ability to impact tumour progression. To test this possibility, it was determined if tumour-generated pressure modifies the bone metastatic niche to promote metastatic growth in a model of prostate cancer (PCa) bone metastasis. Pressure-transducing catheters were implanted into the tibiae of mice and pressure was monitored as tumours developed. Afterwards, osteocytes were subjected to similar pressures *in vitro* to determine the impact on their cytokine expression and the ability of pressure to modify osteocytes impact on PCa growth. PCa tumours growing in the tibiae of mice increased intraosseous pressure above basal levels. Application of similar levels of pressure to osteocytes induced their ability to promote PCa growth and invasion, in part, through up-regulating several chemokines, such as CCL5, and matrix metalloproteinase (MMP) production.
These studies demonstrate that physical forces can alter the tumour microenvironment to promote tumour growth and that osteocytes are important components that promote tumour growth in the bone metastatic niche. Further evaluation of the role of osteocytes and physical forces in tumour progression are warranted and could lead to identification of specific targets to minimize tumour progression.

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P127

LIGHT promotes osteolytic bone metastases in NSCLC patients

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LIGHT is a TNF superfamily member, expressed by activated T cells. It is involved in erosive bone disease, such as rheumatoid arthritis where it stimulates osteoclastogenesis. In multiple myeloma, LIGHT promotes osteolysis by increasing osteoclastogenesis and inhibiting osteoblastogenesis. We investigated whether LIGHT has a role in the osteolytic bone metastatic process induced by non small cell lung cancer (NSCLC), which is a tumor with a marked osteotropism. We analysed by flow citometry the expression of LIGHT on CD4 and CD8 T cells, CD14 monocytes, CD16 neutrophils from peripheral blood of patients and controls. CD8 had a low expression of LIGHT, whereas CD4 expressed it at high level, but without significant differences between the groups. Interestingly, we showed that LIGHT expression was significantly higher in monocytes from bone metastatic patients than non-bone metastatic ones. Since osteoclasts (OCs) originate from monocytes, we studied whether LIGHT interferes with the OC formation *in vitro* by plating PBMCs from NSCLC patients and healthy controls. PBMCs from patients with bone metastases differentiate into OCs without stimulating factors (M-CSF and RANKL), but when we add an anti-LIGHT monoclonal antibody (mAb), we detected a significant dose-dependent inhibition of osteoclastogenesis. For PBMCs of NSCLC patients without bone metastases and healthy controls, we induced osteoclastogenesis with M-CSF and RANKL, and when we add anti-LIGHT mAb, we again reported a significant decrease in OC formation, but only with the highest dose of the mAb, thus suggesting a synergic effect with RANKL. We also dosed LIGHT serum levels without detecting significant differences between patients and controls.
Thus our data demonstrated that the high expression of LIGHT sustains OC formation, suggesting a role of LIGHT in the bone metastatic process from NSCLC.

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P128

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

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Bisphosphonates are used in the treatment of a variety of diseases with skeletal complications. With the development of more potent compounds, there is the

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

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P129

Vertebral fractures among breast cancer survivors in China

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Background
Women with breast cancer (BC) are at high risk for fracture due to the deleterious impact of BC therapies on bone. In China, BC survival is improving as screening and treatment programs expand, however no guidelines exist to prevent BC treatment-induced bone loss. We sought to evaluate the scope of this problem among BC survivors receiving care at a large cancer referral hospital in China.
Methods
Women were invited to participate in this cross-sectional study from 4/2013 to 8/2014. Eligibility criteria included age 50–70 years and initiation of treatment for BC at least 5 years prior to enrolment. Women with bone metastases, or a history of metabolic bone disease prior to BC diagnosis were excluded. Study procedures included a questionnaire regarding risk factors for and personal history of fracture and a thoracolumbar X-ray.
Results
Two hundred women were enrolled with a mean age of 57 ± 5 years and BMI of 24.8 ± 3.7 kg/m^2. Mean years since BC diagnosis was 6.3 ± 1.9. The majority of cases were stage I or II at diagnosis (85.3%) and estrogen and/or progesterone receptor positive (84.6%). In total, 22 vertebral fractures (VFs) were identified. 11% reported a parental history of fracture, 10.5% reported a personal history of fracture, and 15.7% reported falling within the past year. 51% of all participants reported taking calcium supplements, but only 6.1% reported taking vitamin D supplements. Only 27% of women reported having a bone density scan since being diagnosed with BC. Compared with data from an age- and BMI-matched cohort of healthy women from the same city, the odds ratio for vertebral fracture was 3.41 (95%CI 1.42–8.17, $P = 0.006$).
Conclusions
Vertebral fracture risk was high among our cohort of BC survivors. Chinese women undergoing BC therapy should be routinely evaluated for osteoporotic fracture risk. Larger studies are necessary to inform screening and prevention guidelines.

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P130

Uptake of different nitrogen containing bisphosphonate formulations by breast cancer cells

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Nitrogen-containing bisphosphonates (N-BPs) are used to treat osteolytic bone metastases. N-BPs have been previously shown to enter macrophages via macropinocytosis, but the mechanisms how they are taken up by breast cancer cells are so far incompletely known. In breast cancer primary tumours N-BPs have been shown, by other researchers, to be bound to micro-calcifications present in the tumour stroma. In our study we have characterized how different N-BP formulations, free, calcium-bound and liposome encapsulated are internalized to MCF-7 and T47D breast cancer cells.
All of the three different N-BP formulations were shown to utilize the various dynamin dependent endocytosis routes under which the T47D cells used more the clathrin-dependent route and MCF-7 cells a caveolin and clathrin independent route. In both of the cell lines the uptake of ^{14}C-zoledronic acid was enhanced by calcium chelation of the drug (MCF7: free drug 50 c/s per mg protein vs [^{14}C]-Zol-Ca^{2+}2157 c/s per mg protein, $P < 0.001$; T47D: 36.6 c/s per mg protein vs 3368.2 c/s per mg protein, $P < 0.001$). Cytotoxicity was also enhanced by calcium chelation (IC_{50}, MCF7 cells: free drug, 2.3×10^{-4} M vs calcium bound, 4.5×10^{-4} M, $P < 0.05$; T47D cells: 8.97×10^{-4} M vs 7.2×10^{-5} M, $P < 0.001$,respectively) and liposome encapsulation of the drug (MCF-7: 8×10^{-7} M, T47D: 6.9×10^{-6}, $P < 0.001$ for both of the cells lines when compared to the free drug). Liposome encapsulation favoured the use of the dynamin and caveolin dependent endocytosis route. Moreover, liposome encapsulation prolonged the drug effect in cells as monitored by the intracellular formation of isopentenyl pyrophosphate (IPP) and it AMP conjugate ApppI. At 72 h post treatment ApppI was still detected and the IPP concentration was highest in the cells treated with the liposomal drug. These findings suggest that breast cancer cells use different uptake mechanisms for N-BPs than macrophages, which is relevant when cell-specific targeting strategies for N-BPs are planned.

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P131

MicroRNA-30 family regulates bone tropism and osteomimicry in human breast cancer cells

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Breast cancer cells that escape primary tumors disseminate to bone with high affinity at late-stage disease. Circulating breast tumor cells that invade the bone marrow (BM) express sets of deregulated genes and/or microRNAs (miRNAs) that facilitate their bone tropism and enhance engraftment of disseminated tumor cell (DTC) in BM which may subsequently induce osteolytic lesions. MiRNA transcriptomic profiling of the human breast cancer DTC cell line, BC-M1, and of the osteotropic cell line MDA-B02 let us identified drastic down-regulation of the miRNA-30 family (miRs-30), which is composed of five members (miR-30a, miR-30b, miR-30c, miR-30d and miR-30e). Genes encoding for osteotropism and osteomimicry were highly expressed in BC-M1 and MDA-B02 and were predicted *in silico* as being miRs-30 targets. Restoring the miRs-30 expression in MDA-B02 (MDA-B02-miRs-30) inhibited bone metastasis in a murine experimental model of human bone metastasis. Consistent with these *in vivo* data, miRs-30 reduced the expression of osteotropic and osteomimetic genes, *ITGβ3, ITGα5* (Integrin β3, α5), *CDH11* (Cadherin11), *CTGF* (connective tissue growth factor) and *RunX2,* of oncogenic genes*, MTDH* (Metadherin)*, NT5E (*5′-ectonucleotidase*), TNC* (TenascinC) *and NEDD4* (E3-ubiquitin protein ligase) and impacted negatively on tumor cell invasiveness *in vitro*. There was also a substantial reduction of the production of pro-osteoclastic cytokines IL-8 and IL-11 in the conditioned medium (CM) from MDA-BO2-miRs-30 (CM-miRs-30), which was associated with decreased formation of TRAP-positive multinucleated osteoclasts. Additionally, osteogenic properties of MC3T3-E1 osteoblastic cells were inhibited in the presence of CM from MDA-BO2 cells and this inhibitory

effect was repressed by miRs-30 through a decreased production of the Wnt inhibitor *Dkk1*. Moreover, we found that low miRs-30 expression in primary tumors from patients with breast cancer was associated with poor relapse-free survival.
We conclude that miRs-30s impact negatively on BM colonization by breast cancer cells, leading to a reduction in the formation of skeletal lesions in tumor-bearing animals.

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P132

Osteoblast-derived factors increased metastatic potential in human prostate cancer cells

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In prostate cancer TGFβ promotes invasion and metastatic potential. One well-known cellular source of TGFβ in the bone metastatic site is the bone-forming osteoblasts. Here we have studied the effects by human osteoblast-derived factors on metastatic potential in cells from the human bone metastatic prostate cancer cell line PC-3U and the primary prostate cancer cell line 22Rv1. Osteoblast-derived factors resulted in a morphological effect with an increase of long cellular protrusions of the PC-3U cells, an effect dependent on TGFβ-signaling. Also, migration was increased, an effect that was less prominent in PC-3U cells overexpressing a mutated TβRI receptor preventing TRAF6-dependent TGFβ-signaling. Furthermore, osteoblast-derived factors induced loss of cell–cell contacts. The effects of the osteoblast-derived factors on the PC-3U cells were not due to epithelial–mesenchymal transition or neuroendocrine differentiation. The 3D Matrigel-on-top culture method was used for further evaluation of cell characteristics. Interestingly, both 22Rv1 and PC-3U could generate filopodium-like protrusions (FLPs), protrusions previously suggested to be essential for breast cancer metastatis. FLP formation in 22Rv1 was rare, but ten times more frequent in PC-3U. Treatment of PC-3U cells with osteoblast-derived factors, or TGFβ alone, increased the formation of FLPs tenfold. In conclusion, the findings presented here suggest that factors secreted from osteoblasts, including TGFβ, can induce several cellular traits important in metastatic potential of tumor cells, further strengthening the role by bone cells as inducers of metastatic tumor cell behavior.

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P133

Novel evidence that ApoA-1 deficiency facilitates HSC mobilization and differentiation and halts HSC quiescence and self-renewal in mice

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Recent evidence suggests that osteoblastic bone marrow niche is vital for the maintenance and self-renewal of hematopoietic stem cells (HSC). It has been recently proposed that cholesterol efflux pathways participate in HSC mobilization and that cholesterol-sensing pathways control the proliferation of HSC progenitors. Moreover, we have recently documented that HDL perturbations result in impaired osteoblastic function in mice. In the present study, we aimed at investigating the role of ApoA-1, the cardinal regulator of HDL biosynthesis in the regulation of HSC quiescence-mobilization and consequently in hematologic malignancies.

Materials and methods
Whole bone marrow cells (WBMCs) were isolated, from the femora of *ApoA-I*$^{-/-}$ ($n=6$) and WT ($n=6$) C57BL/6 mice and assessed for the expression of factors that are differentially expressed in the BM microenvironment and affect HSC fate. More specifically, we tested the expression of the chemoattractant cytokine CLCX12, its receptor CXCR4, the Jagged-1/Notch (1,2) signaling cascade elements as well as N-cadherin and osteopontin, factors that promote HSC quiescence and self-renewal with qRT-PCR. Additionally, we assessed the expression of CLCX12 and CXCR4 (the most significant regulators of HSC microenvironment) with flow cytometry.
Results
The expression of CLCX12 was significantly reduced, while the expression CXCR4 was greatly augmented (possibly via feedback cell reaction-mechanism) in the WBMC of the *ApoA-I*$^{-/-}$ compared to the WT mice. WBMCs from *ApoA-I*$^{-/-}$ mice displayed strongly decreased mRNA levels of Jagged-1, Notch-1 and -2 and ostepontin. The levels of N-cadherin were greatly elevated in the knock-out compared to the WT animals. In symphony with the RT-PCR results, flow cytometry confirmed the RT-PCR results as regards CLCX12 and CXCR4 expression.
Discussion
The present study suggests for the first time that ApoA-I deficiency halts HSC maintenance and quiescence, whereas it promotes HSC differentiation facilitating the development of hematologic malignancies and possibly bone metastases.

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P134

Encapsulation of Gli-inhibitors blocks tumor invasion into the bone

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It is well established that tumor expression of Gli2, a Hedgehog family transcription factor, contributes to tumor-induced bone disease. Our previous studies investigating genetic inhibition of Gli2 expression in tumor cells have shown promise for the development of therapeutics. While many groups have focused on developing upstream Hedgehog inhibitors for soft-tissue tumors that aberrantly express Gli proteins, we have previously demonstrated that the expression of Gli2 in tumor-induced bone disease is not always regulated by canonical Hedgehog signaling, therefore upstream inhibitors will not be effective. *In vitro* studies have identified several efficacious small molecule Gli inhibitors, but they are hydrophobic and difficult to deliver *in vivo*. We hypothesized that drug delivery strategies using micro/nanoparticles to encapsulate Gli inhibitors would significantly reduce tumor-induced bone destruction. Therefore, we loaded polypropylene sulfide (PPS) microparticles with the Gli2 antagonist GANT58 to inhibit Gli2 locally. The GANT58-loaded PPS microparticles (GANT58-MP) were fabricated using an oil-in-water emulsion method, creating spheroid particles (average size of 4.1 ± 3 μm verified by SEM) that exhibited ROS-regulated controlled release. Since local bony invasion is a serious consequence in invasive oral cancer and Gli2 is overexpressed by these tumors, we initially tested the GANT58-MP in a model of oral cancer invasion into the mandible. Specifically, bony-invasive CAL27 oral squamous cell carcinoma cells were injected into the masseter muscle of athymic nude mice. GANT58-MP treatment significantly reduced bone destruction by greater than twofold ($P=0.0096$). In order to target bone metastatic disease, we have developed similar nanoparticle encapsulation approaches for systemic treatment. Preliminary studies indicate a reduction in lesion area after drug treatment, as well as a significant increase in uptake of the drug in bone with tumors as opposed to non-tumor bone ($P=0.0176$). Thus, these results suggest that targeted Gli inhibitors are a promising reducing tumor-induced bone disease.

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Cell biology: osteoblasts and bone formation

P135

Up-regulation of inhibitors of DNA binding/differentiation gene during alendronate-induced osteoblast differentiation

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Aim
Alendronate enhances bone morphogenetic proteins (BMP)-mediated osteoblast differentiation. A balanced regulation of inhibitors of DNA binding/differentiation (Ids) plays an important role in BMP-induced osteoblast differentiation. However, there are no studies on the possible roles of *Id* genes in alendronate-induced osteoblast differentiation. This study investigated the effect of alendronate on the expression of *Id* genes in osteoblast differentiation.
Methods
C2C12 cells were treated with alendronate for various concentrations and time periods. For evaluation of alendronate-induced osteoblast differentiation in C2C12 cells, alkaline phosphatase (ALP) activity was measured. The expression of osteoblast differentiation markers such as ALP, type-1 collagen (Col 1), and osteocalcin (OCN), and the expression of Id-1 and Id-2 were measured by RT-PCR. In order to understand the mechanism underlying the regulation of *Id* genes, the promoter region of the *Id-1* gene was identified. Database analysis of the promoter region for *Id-1* using known consensus sequences identified several putative response elements, including CCAAT/enhancer-binding protein beta (C/EBPβ).
Results
Alendronate treatment significantly increased not only ALP activity but also expression of ALP, Col 1, and OCN, Id-1 and Id-2. C/EBPβ and alendronate cooperatively increased the promoter activity and expression of Id-1.
Conclusion
These results suggest that C/EBPβ-mediated *Id-1* transcriptional activation may regulate alendronate-induced osteoblast differentiation of C2C12 cells.

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Abstract withdrawn.

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Delta-like 1/fetal antigen 1 (DLK1/FA1) inhibits BMP2-induced osteoblast differentiation by modulating Nfκb signaling pathway: a novel mechanism for regulation of bone formation

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Delta-like 1/fetal antigen-1 (DLK1/FA1) is a negative regulator of bone mass *in vivo* as it inhibits osteoblast (OB) and stimulates osteoclast differentiation. However, the molecular mechanisms underlying these effects are not known. Thus, we studied the effect of DLK1/FA1 on different signaling pathways known to regulate OB differentiation. We identified DLK1/FA1 as an inhibitor of BMP2-induced OB differentiation. Stable overexpression of DLK1/FA1 in C2C12 cells or the addition of its soluble form protein significantly inhibited BMP2-induced OB differentiation evidenced by reduced Alp activity and osteoblastic gene expression. We observed that DLK1/FA1 inhibited BMP signaling by reduced expression of BMP-responsive genes, reduced BMP2-induced luciferase activity in BMP luciferase reporter cells (C2C12BRA), and reduced BMP2-induced Smad1/5/8 phosphorylation. Further studies revealed that DLK1/FA1 affected the expression of both positive (Bmpr1a, Bmpr1b, Bmpr2 and Smad4) and negative (Smad6, Smad7, Smur1 and Smurf2) regulators of BMP pathway. Besides, we observed that DLK1/FA1 induced robust NFκB activity evidenced by NFκB-luciferase reporter assay and real-time RT-PCR analysis of NFκB target genes. Activation of NFκB signaling was found to inhibit BMP signaling in C2C12BRA cells by luciferase assay. In conclusion, we propose a novel mechanism where DLK1/FA1 inhibits BMP2-induced OB differentiation by inhibiting BMP signaling directly through regulation of BMP signaling molecules and indirectly through modulation of NFκB signaling. Our results provide new insight into molecular control of DLK1 on OB differentiation and on bone formation.

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Osteoblastogenesis is regulated through the interplay between human arrest defective 1 and runt-related transcription factor 2

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Arrest defective 1 was originally identified as an acetyltransferase essential for the life-cycle progression in yeasts. Its human orthologue hARD1 has been known to express the enzymatic activity and to acetylate several targets such as HIF-1alpha, MLCK-1, and beta-catenin. Here, whether hARD1 takes part in pre-osteoblast differentiation toward calcium-deposing osteoblast was explored. ALP staining and alizalin red S staining showed that osteoblast differentiation was negatively regulated by hARD1. Using two reporter systems reflecting the transcriptional activity of a runt-related transcription factor, hARD1 was suggested to control osteoblast differentiation by inhibiting the runt-related transcription factor. An immunoprecipitation analyses revealed that of runt-related transcription factors. Runx2 is physically interacted with and acetylated by hARD1. These results support the negative action of hARD1 against the Runx2-mediated osteoblast differentiation. Therefore, it was theoretically expected that hARD1 may be down-regulated during the differentiation because Runx2 should be activated to keep the differentiation. Ironically, the hARD1 expression was noticeably increased while osteoblasts undergo differentiation. Although the precise role of the hARD1-Runx2 interplay in bone formation is not uncovered so far, it could be speculated that hARD1 prevent the overshooting of Runx2 to ensure the harmonious cooperation among numerous osteogenic factors.

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Human bone marrow-derived mesenchymal stem cells response on calcium- and magnesium-ion-implanted resorbable blast media-treated titanium surface

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Purpose
The aims of this study were (1) to assess the topographical and chemical changes of Ca and Mg ion implantation procedures using plasma immersion ion implantation and deposition (PIIID) technique and (2) to evaluate cellular response of human bone marrow-derived mesenchymal stem cells (hBMMSCs) to Ca and Mg ion-implanted titanium surface, compared with resorbable blast media (RBM)-treated titanium surface by observing cell attachment, proliferation and gene expression of the osteoblastic phenotype.

Material and methods
Three different titanium surfaces were analyzed: RBM surface (hydroxyapatite grit blasted), Ca-implanted surface, and Mg-implanted surface. Ca and Mg ion implantation onto surface were performed by PIIID technique. The surface characteristics were evaluated by scanning electron microscopy (SEM), surface roughness tester, X-ray diffractometer (XRD), and Auger electron spectroscopy (AES). hBMMSCs were cultured on three different surfaces for evaluation of cellular response. Initial cell attachment was evaluated by SEM, and MTT assay was used to determine cell proliferation. Real-time PCR was used for quantitative analysis of osteoblastic gene expression (Runx2, Type I collagen, alkaline phosphatase, osteocalcin).
Results
In SEM, surface roughness (Ra) and XRD analysis, there were no changes of surface topography after ion implantation procedure by PIIID technique. In AES depth profile analysis, the concentration of Ca, Mg, oxygen, carbon and titanium varied gradually from the outermost surface to the bulk. Mg ion was present in deeper layers than Ca ion. In cell attachment, Ca- and Mg-implanted surface showed greater quantity and quality of initial cell attachment after 4- and 24-h cultivation. In cell proliferation, there was no statistical difference between three different surfaces. In real-time PCR analysis after 6 days cultivation, expression of Runx2 was higher in Mg-implanted surface and expression of osteocalcin was lower in Ca-implanted surface.
Conclusion
Ca- and Mg-implanted surface showed greater initial cellular attachment. Ion implantation using PIIID technique changed the surface chemistry without topographical change.

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Matrix metalloproteinases and their inhibitors (TIMPs and RECK): induction of osteoblastic differentiation modulates their protein levels on mineralization onset in human dental pulp stem cells

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Constant remodeling of extracellular matrix is a hallmark during physiological conditions, such as stem cell differentiation, embryogenesis and tissue repair. Matrix metalloproteinases (MMPs) play a key role in these processes. MMPs and MMP-inhibitors (TIMPs) are responsible for bone formation and bone matrix remodeling and, probably, determinate the level of its turnover. Mesenchymal stem cells derived from dental pulp are multipotent and have the capacity to differentiate, at least, into mesenchymal tissues, such as bone, fat and cartilage, under inductive conditions *in vitro*. However, few is known about MMPs and their inhibitors role in stem cell biology and the relevance of their gene and protein modulation during osteoblastic differentiation *in vitro*. In this study, we evaluated protein expression of MMPs and their inhibitors (TIMPs and RECK) during osteogenic differentiation induction from DPSCs *in vitro* by western blot. DPSCs ($n=3$) isolated from extracted human third molars were grown in clonogenic medium (α-MEM + 10% FBS + 50 μg/ml ascorbate – used as negative control of differentiation) and differentiation stimulated by osteogenic medium (α-MEM + 10% FBS + 10 mM β-glycerophosphate + 1 μM dexamethasone + 50 μg/ml ascorbate) for 35 days. Several MMPs/inhibitors are expressed by DPSCs and we observed a progressive up-regulation of protein levels from 14 to 35 days after differentiation induction in comparison to their controls. This coincides to onset of mineralization when we detected maximal alkaline phosphatase activity and beginning of calcium nodules staining by alizarin red. Our preliminary results suggest that MMP and their inhibitors may be important to onset to mineralization during osteogenic differentiation *in vitro* from DPSCs.
Keywords: dental pulp stem cells, MMP, TIMP, RECK, osteoblast differentiation, and mineralization.
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P141

Age-dependent changes in the bone marrow microenvironment

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Blood vessels define the properties of local microenvironments in the skeletal system, play crucial roles in osteogenesis and provide niches for haematopoietic stem cells. The properties of niche-forming vessels and their changes in the ageing organism remain incompletely understood. We have previously identified a new capillary subtype in the murine skeletal system with distinct morphological, molecular and functional properties. These vessels are $CD31^{hi}/Emcn^{hi}$, localized to growth plate and endosteal region, 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. Here, we show that Notch signalling in endothelial cells leads to the expansion of haematopoietic stem cell niches in bone. While endothelial hypoxia-inducible factor signalling promotes neo-angiogenesis, it fails to induce arterialization and expansion of PDGFRβ-positive perivascular cells and thereby does not improve vascular niche function. In ageing mice, niche-forming vessels in the skeletal system are strongly reduced but can be restored by activation of Notch signalling in endothelial cells. These findings argue that vascular niches are part of complex, age-dependent microenvironments involving multiple cell populations and vessel subtypes.

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Regulation of genes involved in the integrin signalling pathway induced by titanium with nanotopography

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Titanium (Ti) surface with nanotopography induces osteoblast differentiation, at least in part, by upregulating the expression of α1/β1 integrins. Thus, we hypothesized that other potential candidates from the integrin signalling pathway may be involved in the osteogenic potential of nanotopography. In this context, the aim of this study was to compare the expression of integrin family members of cells grown on nanotopography with machined surface. Machined Ti discs were treated or not with H2SO4/H2O2 to produce nanotopography and the surfaces were analysed by scanning electron microscopy and optical interferometry. Mesenchymal stem cells (MSCs) from rat bone marrow were obtained under the approval of the Committee of Ethics in Animal Research (#2014.1.796.58.7) cultured on both Ti surfaces for up to 7 days. To confirm the osteogenic potential of the nanotopography, the gene expression of some osteoblast markers was evaluated by real-time PCR at day 7. The expression of a panel of genes involved in the integrin signalling pathway was evaluated by PCR array at day 3. All experiments were done in triplicate ($n=3$) and the data were compared by *t*-test ($P\leq0.05$). Ti with nanotopography exhibited a network of nanopits with higher ($P=0.002$) roughness average compared with machined Ti, which presented a smooth surface. The gene expression of Runx2 ($P=0.006$), osterix ($P=0.017$), alkaline phosphatase ($P=0.004$), osteocalcin ($P=0.001$) and bone sialoprotein ($P=0.001$) was higher in cells grown on nanotopography compared with machined Ti surface. The nanotopography induced significant changes (modulation ≥2-fold) in the expression of 12 genes, five integrins and seven members of the focal adhesion kinase signalling pathway ($P\leq0.05$). In conclusion, we have shown that Ti with nanotopography modulates the expression of several genes involved in the integrin signalling pathway at early stages of the culture development, which could be related to the higher osteoblast differentiation induced by this surface.

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P143

RUNX2, osterix and the human sclerostin gene: searching molecular and epidemiological interactions

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Sclerostin, encoded by the *SOST* gene, functions as an inhibitor of the Wnt pathway and thus it is an important regulator of bone homeostasis. The fact that osteoblasts, the only cells expressing *SOST*, lay buried deeply in the bone matrix, poses intrinsic difficulties to the study of the regulation of this gene. Since RUNX2 and SP7/OSX are two known regulators of the differentiation of cells of the osteoblastic lineage, the aim of this study was to determine their potential role in the regulation of human *SOST*.

Chromatin immunoprecipitation experiments performed with osteoblast-like cells expressing sclerostin confirmed that both RUNX2 and OSX bound to the *SOST* promoter in a position closely located to the transcription start site. Using a luciferase reporter system containing the *SOST* promoter region, we showed that both RUNX2 and OSX significantly increased the expression of the *SOST* gene. Interestingly, we also observed an additive effect when cells were transfected with both expression vectors simultaneously. Moreover, when the expression of these genes was measured in human bone samples, we found significant correlations between *SOST* and *RUNX2* expression ($r=0.47$, $P=0.03$), and between *SOST* and *OSTERIX* ($r=0.55$, $P=0.01$) expression, but not between *RUNX2* and *OSX* ($P=0.19$, $P=0.43$). These results further support a crucial role of RUNX2 and OSX in the activation of human *SOST*.

In a genetic association study, two polymorphisms of the promoter region of the *RUNX2* gene were significantly associated with BMD in a group of 987 postmenopausal women ($P=0.02$). However, we did not find statistically significant interactions between them and other *OSX* and *SOST* polymorphisms on bone mineral density.

In summary, the transcription factors encoded by RUNX2 and SP7/OSX bind to the human *SOST* promoter, stimulate *SOST* expression *in vitro* and are likely regulators of sclerostin production in human bone.

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P144

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

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

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

	Fox 1	Fox 2	Fox 3	Fox 4	Fox 5
Sex	Female	Female	Female	Female	Male
Mass (kg)	4.85	4.55	4.9	4.6	5.85
Femur length (cm)	12.5	11.5	12.1	12	13.5

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

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

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P145

Evaluation of release kinetics and mitogenic capacity of collagen barrier membranes supplemented with the secretome of activated platelets

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Success in periodontal regeneration through guided tissue regeneration relies on the healing capacity of the host tissue. Platelet preparations are mitogenic and stimulate regeneration through high growth factor levels. Here we assessed collagen barrier membranes (CBM) as carriers for the secretome of activated platelets. We evaluated cell-attachment, the release of the secretome, and cell proliferation.

Secretome of washed platelets (washed PSEC) and unwashed platelets (unwashed PSEC) was lyophilized on CBM. Then morphology was evaluated by scanning electron microscopy. Cell attachment was measured with fluorescence microscopy based on DiI-labeled cells. To measure the release kinetic we collected supernatants from the loaded CBM. The mitogenic effect was evaluated in a bioassay using fibroblasts of the gingiva and periodontal ligament. Total protein release was assessed using the BCA assay. The release of growth factors was investigated based on PDGF-BB and TGFβ1 ELISAs. To reveal if CBM maintain mitogenic activity cells were seeded onto the washed CBM and proliferation was measured.

The morphology of CBM and cell attachment was not modulated by lyophilizing washed and unwashed PSEC onto the CBM. Supernatants taken after 1 h induced a mitogenic response in fibroblasts and showed the highest total protein, TGFβ1 and PDGF-BB content. The effect on proliferation, total protein, and growth factor release decreased rapidly as observed in subsequent supernatants taken after hour 3, 6, 24, and 48. Supernatants of CBM loaded with unwashed PSEC stimulated proliferation more than supernatants from CBM loaded with washed PSEC. CBM loaded with washed and unwashed PSEC increased proliferation when cells where seeded directly onto the membranes after 48 h of washing.

CBM release growth factors in a burst-like release kinetic with a peak in the first hour and maintain their mitogenic capacity. Taken together our results suggest a two-phasic mitogenic activity of CBM loaded with PSEC.

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P146

Pharmacological activation of the non-canonical TGF-β signaling is a novel strategy to enhance bone formation

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Identifying novel approaches for enhancing osteoblast (OB) differentiation of human skeletal (mesenchymal) stem cells (hMSC) can lead to development of novel anabolic agents required for efficient bone formation. Transforming growth factor-βs (TGF-β1, 2, 3) are one of the most abundant growth factors in bone and play a key role in regulating bone remodeling. Canonical TGF-β signaling inhibits, whereas components of the non-canonical TGF-β signaling (e.g. Akt, P38, ERK) are known to enhance OB differentiation and bone formation. We have identified a small molecule kinase inhibitor H-8, that enhances the *ex vivo* OB differentiation and *in vivo* bone formation by hMSC through activation of non-canonical TGF-β signaling. Adding TGF-β1, 2, 3 to hMSC cultures inhibited OB differentiation, as shown by quantification of alkaline phosphatase (ALP) activity. However, in the presence of H-8, TGF-β 1, 2, 3 enhanced OB differentiation of hMSC. Using Active-site-directed competition kinase binding assay, we show that H-8 inhibited the activity of protein kinase G1, which inhibited activation of RhoA-Akt signaling by TGF-β receptor. Western blot

analysis showed that TGF-β3 treatment of hMSC reduced levels of phosphorylated Akt (S473), whereas in the presence of H-8, TGF-β3 treatment enhanced Akt phosphorylation. In addition, pharmacological inhibition of Akt (using Triciribine) or TGF-β receptor (using SB505124, SB431542) abolished the enhancing effect of H-8 on OB differentiation. Our data demonstrate that pharmacological activation of non-canonical TGFβ signalling is a novel strategy to develop anabolic drugs for enhancing bone formation. H-8 has the potential to be developed into a drug to be used for enhancing osteoblastic bone formation required for treatment of localized bone defects.

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P147

Skin-derived IL-17A induces bone loss in the absence of adaptive immunity

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Inflammatory stimuli can lead to bone loss by mechanisms that are not well understood. We recently showed that skin inflammation induces bone loss in mice and humans. In psoriasis, one of the prototypic IL-17A-mediated inflammatory human skin diseases, low bone formation and bone loss correlates with increased serum IL-17A levels. Similarly, in two mouse models with chronic IL-17A-mediated skin inflammation, *K14-IL17A^{ind}* and *JunB$^{\Delta ep}$*, strong inhibition of bone formation occurs, different from classical inflammatory bone loss where osteoclast activation leads to bone degradation. We show that under inflammatory conditions skin resident cells, such as keratinocytes, γδ T cells and innate lymphoid cells express IL-17A, which act systemically to inhibit osteoblast and osteocyte function by a mechanism involving Wnt signaling. IL-17A leads to decreased Wnt signaling *in vitro* and, importantly pharmacological blockade of IL-17A rescues Wnt target gene expression and bone formation *in vivo*.
To determine the contribution of T-cell-derived IL-17A to skin inflammation and bone loss, we crossed the *JunB$^{\Delta ep}$* mice to *Rag1$^{-/-}$* (DKO) mice. Interestingly, we observed exacerbated skin inflammation and earlier onset of bone loss. IL-17A expression was highly abundant in the skin of the DKO mice with increased infiltration of innate immune cells, such as neutrophils and mast cells. These mice were also prone to *S.aureus* infections. Antibiotic treatments reduced skin inflammation and bone loss. We are currently investigating the IL-17A producing cells in the skin in the absence of mature B and T cells, and their possible mobilization to the bone by using the *IL-17A-GFP* reporter mouse. Metagenomic sequencing of the skin microbiota, as well as germ-free housing of these mice, will allow us to determine the role of different commensal bacteria populations in skin inflammation and bone loss. This study adds the dimension of the role of microbiota to the current understanding of osteoimmunology.

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P148

Cationic nacre ethanol soluble matrix has an osteoanabolic effect on human subchondral osteoarthritic osteoblasts and MC3T3-E1 cell line

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Nacre is able to induce bone-forming cells mineralization, and gains widespread interest in bone regeneration. While, the osteoinductive compounds are not identified yet. The nacre extract, ethanol soluble matrix (ESM), was proven having the capacity to induce the mineralization of mouse MC3T3-E1 cells and to restore mineralization defect in human osteoarthritic osteoblasts. Being tested on the two cells, ESM could induce the formation of mineralization nodules, identified as crystalline hydroxyapatite by RAMAN spectroscopy, and stimulate the expression of bone markers, collagen type I, Runx2, osteopontin and osteocalcin. Besides, the formation of collagen fibres in extracellular matrix were also observed with SHG imaging. Thus, ESM is suitable to serve as a source of osteoinductive compounds.
Recently, a new step was done in the purification of the active nacre compounds to the way of the identification. By using ion-exchange resin and cation exchange HPLC, cationic ESM (ESMc), anionic ESM (ESMa) and ten cationic HPLC fractions of ESM were achieved and tested to validate their osteoanabolic activity. ESMc and 2 HPLC fractions stimulated the mineralization in both cell types. No interaction on bone mineralization between ESMc and ESMa was observed. Energy dispersive X-ray spectrometry demonstrated the abundant presence of calcium and chloride in the osteogenic fractions. To validate, pure $CaCl_2$ was tested and proven having an osteogenic effect in both cells, but less stable than ESM, suggesting that free Ca plays a role, though limited, in inducing the cell mineralization.
In conclusion, ESM has an osteoanabolic effect, which may bring some innovative therapeutics for hypomineralization diseases, such as osteoporosis. The cationic ESM, a purified matrix of ESM, makes the identification prospective a bit closer.

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P149

Enhancement of bone ultra structure preservation using high-pressure freezing and microwave-assisted fixation

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Despite the improvement in bone's molecular, metabolic and live-cell imaging, histological investigation remains most crucial in bone biology for diagnostic and research purposes. Therefore, fixation of bone samples – especially for electron microscopy investigation – is critical to the ultrastructural analysis. Up to date, chemical fixation of bone tissue is performed at room temperature resulting in a compromised ultrastructure of bone sample. In this study, we aim at achieving a close-to-native preservation of bone tissue ultrastructure to enhance the understanding of cell–cell and cell–matrix interaction, mineral composition correlation, and three-dimensional organization.
Young male rat bone samples (femora and spine) were fixed using new methods; microwave-assisted chemical fixation (MCF), and high-pressure freezing (HPF) followed by freeze substitution. Furthermore, HPF was used to fix bone marrow aspirate of rat tibia and reaming debris of a 76-year-old male patient.
Large bone samples showed improved ultrastructure and bone matrix preservation using the MCF method compared to conventional fixation. The method enhanced preservation of fibrillar structure organization, octeocytes, fibroblasts and osteoclasts and allowed better identification of their bilayer-membranes and organelles like mitochondria, lysosomes, nuclei and vesicles. Interestingly, unlike conventional protocols fat cells were vastly well preserved. Bone marrow-aspirate as a liquid sample is challenging to fix, however, using HPF achieved excellent preservation shown for the first time. Megakaryocytes exhibited highly homogenous-cytoplasm and were evenly stained. At high magnification of electron microscopy megakaryocytes cytoplasm appeared dens involving regularly rounded organelles like mitochondria, nuclei and vesicles as well as rough endoplasmic reticulum. MCF has shortened the process of bone samples preparation for TEM investigation and allowed better fixatives penetration improving ultrastructure preservation. The processing enhanced the fixation steps such as fixative-crosslinking, dehydration, infiltration of embedding medium and polymerization. Interestingly, HPF processed samples exhibited a close to genuine ultrastructure preservation of bone marrow as a unique sample type.

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P150

GPR39 is negatively regulating osteoblast differentiation and bone formation during aging

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G-protein coupled receptors (GPCRs) are the largest family of cell surface proteins, which are important for bone development, remodeling and diseases. One of the GPCRs, called GPR39, has been found to be expressed in several osteoblastic cell lines. However, its role in bone metabolism has not been investigated yet. In order to elucidate a role for GPR39, we characterized the bone phenotype in GPR39 deficient mice. During aging, at six months old, dynamic histomorphometry data reveal significant increase of mineralizing surface and

bone formation rate in GPR39 deficient mice compared to wild type mice. Furthermore, we show by micro CT that bone mineral density of trabecular bone of femurs in GPR39 deficient mice is also significantly increased. Goldner's Trichrome staining of undecalcified bone sections show significant increase in osteoblasts number and osteoid surface in GPR39 deficient mice, explaining higher rate of mineralization. Level of bone formation biomarker PINP is also significantly higher in GPR39 deficient mice, indicating an increase of bone formation. However, level of CTX-1 biomarker for bone resorption was significantly lower in GPR39 deficient mice. On the other hand, TRAP analysis show normal osteoclasts number and osteoclasts size in GPR39$^{-/-}$ bone sections. In vitro, bone marrow derived mesenchymal stem cells were differentiated into osteoblasts. Stainings of osteoblast culture show imbalance between bone matrix formation and mineralization, where Alizarin Red staining was higher and Collagen I staining lower in GPR39 deficient osteoblasts compared to wild type osteoblasts. mRNA expression analysis show enhanced expression levels of osteoblasts and osteocytes regulating genes in GPR39 deficient osteoblasts, suggesting that GPR39 negatively regulates osteoblast differentiation.
Our data reveal a novel role for GPR39 in regulation of bone metabolism and suggest that absence of GPR39 leads to enhanced bone formation.

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P151

Early deletion of menin in the osteoblast lineage results in decreased bone mass in adult mice

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Menin, the product of the Men1 tumor suppressor gene, facilitates the cell proliferation control and differentiation induced by the TGF-beta superfamily of ligands critical for bone development and maintenance. Our in vivo studies have shown the importance of menin for proper functioning of the mature osteoblast and maintenance of bone mass in adult mice. In the present study, we examined the in vivo role of menin at earlier stages of the osteoblast lineage through conditional knockout of the Men1 gene. This was implemented through the Cre-LoxP recombination system at the level of the osteochondroprogenitor, osteoblast progenitor as well as, for comparison, the mature osteoblast. Prx1-Cre;Men1f/f, Osx-Cre;Men1f/f and OC-Cre;Men1f/f mice represent knockout of the Men1 gene in the mesenchymal stem cell, the preosteoblast and the mature osteoblast, respectively. Mice were sacrificed and analyzed at six months of age. All experiments had institutional ethical approval. Prx1-Cre;Men1f/f and Osx-Cre;Men1f/f mice were smaller than wild-type littermates whereas OC-Cre;Men1f/f mice were of normal size. Femur lengths of Prx1-Cre;Men1f/f and Osx-Cre;Men1f/f mice were shorter whereas those of OC-Cre;Men1f/f mice were of normal length. Prx1-Cre;Men1f/f and Osx-Cre;Men1f/f mice had reduced BMD (dual energy X-ray absorptiometry) whereas that of OC-Cre;Men1f/f mice was normal. By 3-dimensional micro-CT imaging of femur, all three strains of Men1 knockout mice had decreased trabecular bone volume with altered trabecular structure and decreased cortical bone thickness. In all strains of knockout mice trabecular number and spacing were decreased and increased, respectively. Primary calvarial osteoblasts of all strains of knockout mice relative to those of wild-type mice were deficient in mineralization and differentiation as assessed by Alizarin red, von Kossa and alkaline phosphatase staining, and had impaired responsiveness to TGF-beta and BMP-2 in specific promoter-reporter transfection assays. In conclusion, menin plays a crucial role in the development as well as maintenance of bone mass.

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P152

Participation of microRNA-34a/RANKL in the osteogenic potential of the Poly(vinylidene-trifluorethylene)/barium titanate membrane

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Barrier membranes have been extensively used in dentistry to prevent soft tissue down-growth into the bone defects and to promote alveolar ridge augmentation. Previous studies of our group showed that the Poly(vinylidene-trifluoroethylene)/barium titanate composite (PVDF) enhances both the *in vitro* osteoblastic differentiation and the in vivo bone repair compared with a commercially available polytetrafluoroethylene (PTFE) membrane. As bone formation may be regulated by post-transcriptional events such as temporary expression of microRNAs (miRs), the aim of this study was to investigate a possible mechanism involving miRs and RANKL in the osteogenic potential of the PVDF membrane. Rat calvarial bone defects were implanted with either PVDF or PTFE membrane, under the approval of the Committee of Ethics in Animal Research. At 4 and 8 weeks, the new-formed bone was submitted to a large-scale analysis of miRs by microarray, followed by the evaluation of the expression of miR-34a (microarray validation) and RANKL, one of its targets, by quantitative real-time PCR. Also, histochemical analysis was carried out to detect TRAP positive cells. All quantitative data were obtained in triplicate ($n=3$) and compared by t-test ($P\leq0.05$). Among 250 evaluated miRs, miR-34a, -10a and -133b were simultaneously upregulated (>2 fold) at 4 and 8 weeks. At 8 weeks, the expression of miR-34a was higher ($P=0.016$) in the bone grown on PVDF compared with PTFE, followed by a downregulation of the RANKL expression ($P=0.004$). In addition, more TRAP positive cells were observed on new-formed bone grown on PTFE compared with PVDF membrane in both periods. In conclusion, PVDF membrane induces higher bone repair, at least in part, by triggering an intracellular mechanism of miR-34a upregulation/RANKL dowregulation loop, which inhibits osteoclastic activity.

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RhoA and ROCK regulate spreading of osteoblasts

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When cells detach from or re-attach to the substrates, there is a drastic change in shape. Molecular mechanism by which controls shape upon cell attachment to the substrates was studied in osteoblastic MC3T3-E1 cells *in vitro*. Most striking feature found in the ovoid cells detached from the substrates was densely packed cortical actin bundles (CAB) encircling cells. Cells lost the CAB upon attachment and spreading on to the substrates. CABs were under control of small GTPase RhoA (RhoA)/Rho-associated kinase (ROCK) signaling. Cells less spread showed a higher RhoA activity as well as richer CAB on the substrates of lower adhesion strength. Inhibition of adhesion signaling by FAK inhibitor showed the same feature of cells seeded on to the substrates of lower adhesion strength. However, detaching cells of which RhoA or ROCK activity was inhibited maintained their spreaded shape of attached cells without developing CAB. On contrary, attaching cells of which RhoA activity was stimulated by overexpressing constitutively active RhoA maintained ovoid shape of the detached cells. Furthermore, cells spreading on the substrates activated Rac1. Increase of Rac1 activity was also clear in the spread cells which were induced by RhoA-ROCK inhibition on the substrates of low adhesion strength. However, cells overexpressing dominant negative Rac1 did not spread despite of RhoA-ROCK inhibition. Furthermore, without chemico-mechanical improvement of the substrate condition, Rho-ROCK inhibition enhanced the cell attachment and spreading on to the substrates of which adhesion strength is extremely low. These results indicate that inhibition of RhoA-ROCK by adhesion signaling makes cells spread by eliminating contraction force of the CAB and activating Rac1. Rho-ROCK inhibition can be a way to enhance the cell attachment and spreading on to the substrates for the bone tissue engineering which generally uses hydrophobic scaffolds of much low adhesion strength as carriers for cells.

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P154

Low adhesive scaffold collagen promotes the osteogenic differentiation of rat marrow mesenchymal cells

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Background
Collagen has biocompatibility and biodegradability with tissue or organ, therefore, collagen is the most promising material for tissue engineering. In particular, the binding of collagen to specific cells is considered an essential function to develop scaffolds. However, in some cases the binding inhibits the cell motility. In addition, it is not clear whether collagen is the effective scaffold to promote osteogenic differentiation. These days, we succeeded in developing low

adhesive scaffold type I collagen (LASCol) (patent pending) which has the ability to form fibrils. In this study, we report that LASCol markedly facilitates osteogenic differentiation of rat marrow mesenchymal cells (rMMCs).
Methods
The culture dish was coated with LASCol or atelocollagen. Subsequently, rMMCs (5×10^4 cells/dish) were cultured on each coated-dish with osteogenic basal medium. To evaluate osteogenic differentiation, we monitored the differentiation of rMMCs by alkaline phosphatase (ALP) staining and investigated the mineralization by Alizarin Red S (ARS) staining. Furthermore, we measured Gla-Osteocalcin in culture medium by ELISA kit.
Results
Rat MMCs on LASCol coated-dish formed spheroid bodies in 1 day culturing. Each cell of spheroids highly expressed alkaline phosphatase activity. At the same time, the quantity of calcium deposition increased in the conditions of LASCol culture. In contrast, ALP activity and ARS staining of rMMCs on atelocollagen coated-dish were very similar to those of non-coated control dish. Gla-osteocalcin in the culture medium of LASCol was shown to significantly increase. Thus, we demonstrated that LASCol has the potential to induce osteogenic differentiation of rMMCs.
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P155

L-Carnitine facilitates mitochondrial activity and osteogenic differentiation in human primary osteoblast culture

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Osteoblasts have a high rate of energy consumption during bone formation and bone protein synthesis. Therefore, it is likely that decreased energy production that accompanies aging, could contribute to reduced osteoblast activity, a critical feature of senile osteoporosis, and that this reduction might be counteracted by favoring energy availability. Cells of the osteoblastic lineage generate 40–80% of the requested energy through fatty acid degradation, thus the modulation of lipid oxidation could be involved in the availability of energy required for protein synthesis. L-Carnitine (LC) promotes energy availability, as it is an essential cofactor for the transport of fatty acid into the mitochondrial matrix where β-oxidation occurs. Based on these evidences, we studied the possible stimulatory effects of L-Carnitine on cells of the osteogenic lineage. For this purpose we treated human osteoblasts (hOb) derived from trabecular bone waste material of orthopaedic surgery (Ethics Committee approved), with 5 mM LC at different times,3,6 and 24 h.
After 24 h treatment LC induced an increase in mitochondrial activity, as shown by Mito Tracker Dye assay. This effect was accompanied by a significant increase of SOD2 protein levels, which plays an important role in neutralizing oxidative stress, and an increase in Calcium-Calmodulin Kinase II protein (CaMKII). Since CaMKII is able to favour synthesis and transcriptional activity of osterix (OSX), we evaluated the effect of LC on osteogenesis-related genes. LC increased significantly ($P<0.01$) OSX and bone sialo-protein mRNA expression. Moreover, LC stimulated osteopontin mRNA and protein expression. Altogether these results suggest that LC, exerts a stimulatory effect on bone by favouring osteoblast differentiation. Considering its safety profile, LC could be a useful supplement to support bone health in the elderly.

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P156

The differential effects of zoledronate and etidronate on the *in vivo* early osseous healing process of an extracted socket and a tibial defect

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Bisphosphonates (BPs) are widely used for treating osteoporosis and preventing osseous metastasis or hypercalcemia in cancers. However, osteonecrosis of the jaw in patients treated with BP after dentoalveolar surgery has been increasing, especially in those treated with strong BPs such as zoledronate (ZA). The pathobiology underlying the occurrence of osteonecrosis only in the jaw bone remains unclear. The objective of this study was to compare the effects of BPs on the *in vivo* osseous healing process between an extracted socket and a tibial defect. Rats were intravenously injected with 0.067 mg/kg of ZA, the weaker BP etidronate (EA), or vehicle once a week for six weeks. A mandibular molar was extracted, and a hole defect was created in the tibia 2 weeks after BP treatment. Bone healing was evaluated using microcomputed tomography and histological staining 1 and 4 weeks after defect creation. Pre-administration of ZA inhibited bone resorption at both extraction molar and tibia defect. The subsequent net result was impaired bone healing at the extraction socket versus excessive woven bone formation in the tibia. The healing process involved bone resorption before bone formation in the socket, whereas the opposite was true for tibia defect. However, the EA enhanced the *in vivo* bone healing of both areas and had minor anti-resorptive activity as like control. The activity on osteoclast activation *in vivo* was consistent with the *in vitro* effects of the two BPs on osteoclast differentiation. In conclusions, the anti-resorptive action of ZA had a negative effect on bone healing, particularly at the extraction socket in the jaw. These results suggest that the difference in healing process is another critical factor to the preferential osteonecrosis at the area of extracted teeth rather than tibial defect after pre-administration of a potent anti-resorptive BP.

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P157

Evidence for an osteogenic activity of betaine in human osteoblast in culture

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Betaine (BET) is a component of many food. It is an essential osmolyte and a source of methyl groups. BET consumed from food sources and through dietary supplements presents similar bioavailability. BET exerts an antioxidant activity and decreases inflammation states. Dietary supplement with BET are used in many inflammation-connected pathologies, although its mechanism of action is not fully understood. Recent studies have shown that BET stimulates muscle fibers differentiation via insulin like growth factor 1 (IGF-I). It is well known that IGF-I is one of the proliferating and differentiating factors affecting osteoblast activity. IGF-I and II are the most abundant growth factors produced by bone and stored in the bone matrix. On this basis, we evaluated the effect of BET on bone cells. Human osteoblasts (hOb) derived from trabecular bone waste material of orthopaedic surgery (Ethics Committee approved) were treated with 10 mM BET at 3, 6 and 24 h.
Real-time PCR results showed that BET induced a significant increase in IGF-I mRNA at 3 ($P<0.05$) and 6 h ($P<0.01$) after treatment. Immunofluorescence staining showed a significant increase of IGF-I protein at 6 and 24 h after BET treatment. Moreover, BET stimulated significantly ($P<0.01$) the expression of RUNX2, osterix, bone sialo protein and osteopontin. Western blotting results showed a significant ($P<0.05$) increase of osteopontin as well. BET was also able to increase the expression of SOD1 mRNA and SOD2 protein levels.
These preliminary results showed that BET has a stimulatory effect on osteogenic genes and on genes involved in antioxidant activity in hOb. Further studies are needed to fully characterize the mechanisms of action of BET in bone. However, considering that senile osteoporosis is frequently accompanied by enhanced inflammatory state, there might be a rationale in the use of BET as dietary supplement.

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P158

A single 2-day pulse of activin-A leads to a transient change in gene expression eventually followed by reduction in extracellular matrix mineralization

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Activins belong to the transforming growth factor-β superfamily, and they regulate bone formation by controlling both osteoclast and osteoblast behaviour. We have previously shown that activin-A strongly inhibited matrix mineralization in osteoblast cultures, and that activin A-signalling was most effective before the onset of mineralization.
The aim of this study was therefore to investigate how an early activin-A pulse affected osteoblast mineralization and gene expression profile, to unravel the molecular mechanisms of activin-A signalling.
Human osteoblasts (svHFOs) were treated with a 2-day-pulse of activin-A, and mineralization and gene expression profiling have been analysed up to 10 days later.
Our results showed that a single pulse of activin-A at day 5 of culture was sufficient to significantly reduce matrix mineralization at later stages of osteoblast differentiation ($P<0.001$). Activin treatment led to a transient peak (1 h) in SMAD3 phosphorylation, as assessed by Phospho Flow Cytometry. A single pulse of activin-A is therefore responsible for changes at later stages of cell differentiation. Gene expression profiling showed that the activin A-pulse induced a transient change in osteoblast gene expression, in a 2-phase fashion over time: first phase (1–8 h after activin A-pulse), second phase (1–2 days). During the first phase, 38 genes were differentially regulated ($P<0.01$). These changes were mainly related to transcription regulation and some of these transcription factors contained SMAD-responsive elements. In the second phase, 65 genes were differentially regulated ($P<0.01$), and they were mainly involved in ECM and cell-matrix adhesion.
In summary, these findings give new insights into the mechanism by which activin-A modulates osteoblast differentiation, by influencing gene transcription and leading then to alterations of matrix mineralization. Modulation of activin A-signalling might be useful for therapeutic purposes to control bone formation and mineralization and thereby quality.

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P159

Osteogenic superiority of bone marrow-derived osteoblastic cells (ALLOB®) over bone marrow-derived mesenchymal stromal cells

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Bone therapeutics is a bone cell therapy company addressing high unmet medical needs in the field of bone fracture repair, more specifically in non-union and delayed-union fractures where the bone repair process is impaired. The company has developed a unique allogeneic osteoblastic cell product (ALLOB®) derived from bone marrow which is currently tested in humans in the indication of delayed-union fractures. The purpose of the study was to directly compare ALLOB® vs non-differentiated mesenchymal stromal cells (MSC) for their *in vitro* osteogenic characteristics and their *in vivo* osteogenic potential in order to determine which cellular type would be the most adapted for bone fracture repair.
Methods
Healthy volunteers' bone marrow aspirates ($n=6$) were expanded (i) into BM-MSCs using a complete MSC culture medium or (ii) into ALLOB® cells according to its manufacturing process. Cells were characterized *in vitro* by morphology, immunophenotype, gene expression using RNAseq technology and their differentiation potential. Additionally, their osteogenic potential was assessed *in vivo* in the calvaria bone formation model in nude mice ($n=6$ per group).
Results
The *in vitro* side-by-side comparison studies showed that although ALLOB® and BM-MSC shared some common general characteristics such as the three minimal MSC Dominici's criteria, ALLOB® expressed significantly higher levels of chondro/osteoblastic genes such as *BMP2* (fold change (FC) >100), *ALPL* (FC>12), C*BFA1* (FC>3) and differentiated significantly earlier than BM-MSC toward the osteogenic lineage. Moreover, the bone formation model in nude mice demonstrated that used at the same cellular concentration, ALLOB® was able to induce significantly more (160 vs 107%) bone formation than BM-MSC (118 vs 107%) only 2 weeks after administration.
Conclusion
Our side-by-side comparison studies demonstrated that *in vitro* and *in vivo*, ALLOB® displays superior osteogenic capacity to BM-MSC and is therefore a better candidate for the treatment of bone fractures.

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P160

Effect of melatonin on *in vitro* bone remodelling and blood supply

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Melatonin is a neuro-hormone released primarily from the pineal gland, which has been shown to have bone anabolic effect, although it is still unclear whether its skeletal action is directly mediated by receptors expressed on bone cells or is indirect. In this study, we examined melatonin's effects on bone cellular activities *in vitro* and tested whether it modifies angiogenesis and blood flow to bone, which are both essential for bone formation.
Primary osteoclasts from mouse bone marrow cells and osteoblasts from mouse calvariae were cultured in increasing concentrations of melatonin (0.1–100 μg/ml) to evaluate its effect on bone cell numbers and activities. RNA was extracted from mouse primary osteoblasts and osteoclasts for detection of melatonin receptors MT1 and MT2 using RT-PCR.
Bone blood flow of the hind limb was measured by laser Doppler imaging after intraperitoneal injection of melatonin in C57Bl6 mice (1 and 10 mg/kg, $n=5$). The effect of melatonin (1 100 μg/ml) on angiogenesis was assessed using the chick chorioallantoic membrane assay (CAM).
Both MT1 and MT2 receptors were expressed in mouse primary osteoclasts and osteoblasts. Quantitative RT-PCR showed a higher expression of MT2 in osteoblasts, however MT1 was more expressed in osteoclasts. Results show that melatonin dose-dependently increased bone nodule formation from 10 μg/ml ($P<0.0001$). Melatonin had no significant effect on osteoclast number and bone resorption activity *in vitro*. *In vivo*, melatonin did not affect the hindlimb blood flow in C57BL6 mice compared to control and did not stimulate neovascularisation *in vitro*, using the CAM assay.
In conclusion, our results confirm that melatonin is a potent bone anabolic *in vitro* and suggest that its skeletal effect could be directly mediated by MT2 expressed in osteoblasts rather than by increasing bone blood supply.

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P161

Vaspin regulates the osteogenic differentiation of MC3T3-E1 through the PI3K-Akt/miR-34c loop

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Vaspin (visceral adipose tissue-derived serine protease inhibitor) is a newly discovered adipokine that widely participates in diabetes mellitus, polycystic ovarian syndrome and other disorders of metabolism. However, the effect of vaspin on the regulation of osteogenesis and the mechanism responsible are still unclear. Here, we found that vaspin could attenuate the ALP activities, osteocalcin secretion and Runx2 expression in the preosteoblast cell line MC3T3-E1 in a dose-dependent way, which suggested vaspin inhibit the osteogenic differentiation of MC3T3-E1 cells; also, during this process, the expression of miRNA-34c (miR-34c) was significantly increased confirmed by miRNA microarray and real-time PCR. Down-regulation of the expression of miR-34c in MC3T3-E1 cells diminished the osteogenic inhibitory effect of vaspin, while the up-regulation of miR-34c increased this effect. Using luciferase reporter assays, we confirmed Runx2 is the target of miR-34c. Meanwhile, we found that vaspin could up-regulation of miR-34c through activate the PI3K-Akt signalling pathway. Blocking the PI3K-Akt signalling pathway with specific inhibitors could decrease the expression level of miR-34c, meanwhile the osteogenic inhibitory effect of vaspin also decreased. Furthermore, knock-down of miR-34c could promote the activation of Akt, which was realised by decreased c-met expression, which was the target of miR-34c and the upstream of Akt. Thus, PI3K-Akt, miR-34c and c-met constituted a modulation loop and controlled the expression of each other. Taken together, our study showed that vaspin could inhibit the osteogenic differentiation *in vitro*, and the PI3K-Akt/miR-34c and miR-34c/c-met loop might be the underlying mechanism.

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P162

RCOR2 is a novel regulator of osteoblast differentiation

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Epigenetic mechanisms regulating osteoblast differentiation are still inadequately described. In a genome wide transcriptional profiling of MC3T3-E1 osteoblastic cell line, we identified RCOR2 as a significantly upregulated gene during a differentiation time-course from proliferative to mature osteoblasts. Similar expression profile of RCOR2 was found in mouse calvarial osteoblasts. RCOR2 belongs to CoREST/RCOR family of proteins that regulate action of lysine-specific histone demethylase 1 (LSD1). LSD1 controls gene expression, for example, by demethylating histone H3K4me1/2, leading generally to gene repression. Lentiviral shRNA-mediated silencing of RCOR2 expression in MC3T3-E1 cells led to impaired osteoblastic differentiation shown by decreased ALP staining and number of mineralized bone nodules, and reduced expression of osteoblast-related genes. LSD1 is abundantly expressed in MC3T3-E1 cells and we showed that RCOR2 and LSD1 co-immunoprecipitated in HEK293T cells. However, we did not find differences in global H3K4me1/2 levels between shRCOR2 and control cells. To identify specific genes and pathways associated with RCOR2 action, we performed RNA-seq analysis of MC3T3-E1-shRCOR2 cells at the early stages of differentiation. GO pathway analysis showed upregulation of genes related to cellular metabolism and embryonic morphogenesis in shRCOR2 cells, suggesting RCOR2 to repress these pathways at the onset of differentiation. Using our genome-wide H3K4me1 ChIP-seq map of MC3T3-E1 cells, we identified putative enhancer regions in selected shRCOR2-affected genes and tested them for H3K4me1 occupancy in shRCOR2 and control cells by ChIP. We observed alterations in H3K4me1 levels relative to total histone H3 occupancy in RCOR2 knockdown cells, suggesting changes in LSD1 activity at specific genomic sites. In conclusion, RCOR2 is an important new player in regulating the transition from proliferative to committed osteoblastic phenotype. Our data suggest that RCOR2 could function at least in part via regulating histone methylation and provide important new information on the epigenetic regulation of chromatin landscape during osteoblast differentiation.

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Inhibition of histone demethylase LSD1 suppresses osteoblast differentiation

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Post-translational modifications of histone N-terminal tail domains affect the local chromatin conformation and serve as a dynamic regulatory layer for controlling gene transcription during cell differentiation. However, the role of epigenetic modifications in the regulation of osteoblast differentiation is still not fully understood. Recently, we identified RCOR2 as a novel regulator of osteoblast differentiation in a genome-wide transcription profiling of mouse MC3T3-E1 cells and showed that shRNA-mediated silencing of RCOR2 impairs osteoblast differentiation. RCOR2 is a close homolog of RCOR1 (CoREST), which forms a complex with lysine-specific demethylase 1 (LSD1) and regulates its activity and function. LSD1 has been shown to regulate embryonic development and cellular differentiation. We previously confirmed that RCOR2 and LSD1 physically interacted, which led us to hypothesize that the osteoblastogenesis-promoting effect of RCOR2 could be mediated by LSD1. Inhibition of LSD1 activity with micromolar concentrations of small molecular inhibitors S2101 and RN-1 resulted in impaired differentiation of MC3T3-E1 cells and mouse calvarial osteoblasts, shown by decreased ALP staining and decreased expression of osteoblast-related genes including ALP, OSX and OCN. S2101 and RN-1 did not show toxicity for osteoblasts at used concentrations. We found that LSD1 inhibition in MC3T3-E1 cells resulted in altered expression of a set of genes that we had previously identified to be regulated by RCOR2, further supporting the hypothesis that RCOR2 and LSD1 function in the same pathway to regulate common targets in osteoblastic cells. In conclusion, our results indicate that LSD1 is involved in the regulation of osteoblast differentiation. As inhibition of LSD1 resembles closely the effect of RCOR2 downregulation, they act likely as components of the same control node in the epigenetic regulation of osteoblast differentiation. Studying the possible bone-related effects of LSD1 inhibition is highly clinically relevant due to the recent interest of LSD1 as a drug target in oncology.

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P164

Natural uranium triggers autophagy in osteoblasts

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Bone is a complex organ constituted of a mineralized matrix generated by osteoblasts (OB). Bone matrix is a major storage site for minerals but also for toxicants from the environment. Among them, uranium, a natural element of the earth crust, has a dual toxicity due to its radiological effects as an alpha emitter and its chemical effects due to its metal properties. In the case of natural uranium, the chemical toxicity is predominant. Uranium level in drinking water is usually in the range of microgram-per-liter but this value may be as much as 100 to 1000 times higher in some geographical areas and the effects of a chronic exposure on bone biology have been poorly explored. Using an osteoblastic cell line, we have shown that the presence of natural uranium, even at low and non-toxic concentrations, alters the main OB function i.e. matrix mineralization. A 24 h OB exposure to sub-toxic uranium level results in uranium precipitate formation which can be observed within autophagic vacuoles, multivesicular bodies, lysosomes as well as in the extracellular space. In addition, natural uranium triggers autophagy in OB after a 3 h exposure, with a block of the autophagic flux observed after a 24 h exposure. These results indicate that autophagy is activated in response to uranium in OB.

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P165

Canine osteoblasts from trabecular, cortical and subchondral bone present differences in alkaline phosphatase activity

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

Femoral heads from three canine hip replacement surgeries were collected. Fragments of bone from subchondral, trabecular and cortical areas of the femoral epiphysis were collected and washed in PBS + AB/AM, trypsin-digested and incubated in 0.2% collagenase. The fragments were seeded in DMEM + 10% FCS + AB/AM at 37 °C, 5% CO_2, grown until confluence and the cells seeded ($n=6$) at a density of 1.3×10^4 cells/cm^2. We determined cell proliferation after 4 days with crystal violet staining and basal alkaline phosphatase activity 24 h post-confluence.

No significant differences were found in cell proliferation or doubling time among bone types, however basal alkaline phosphatase activity was higher in trabecular than in subchondral ($P<0.05$) or cortical ($P<0.001$) bone cells. We are now optimising culture conditions to stimulate matrix mineralisation activity.

Our preliminary data indicates that canine osteoblast activity differs between trabecular, subchondral and cortical bone types. This could have significant

implications for future design of implants that osseointegrate leading to improved longevity and faster recovery from hip replacement surgery.

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P166

Primary osteoblast culture from domestic dog (Canis lupus familiaris)

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

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

	Mass (Kg)	Sex	Age (years)	Breed
Canine 1	39	Male	5.53	German shepherd
Canine 2	33	Female	8.85	Labrador
Canine 3	30	Female	1.00	Briard

Median cell number reached 52.5×10^3 [$30.1 - 72.5 \times 10^3$] cells/cm^2 with doubling time of 3.5 [1.6 − 4.9] days. We found that cells from the 1 year-old Briard had greater cell count and shorter doubling time than the 8 year-old Labrador ($P < 0.001$ for both cases). TNAP activity at day 1 post-confluence was increased in the Labrador sample when compared to the 1 year-old Briard (0.01 [0.01 − 0.02] *vs* 0.03 [0.02 − 0.04] U/min per mg protein; $P < 0.001$). This difference wasn't found at subsequent time-points since TNAP activity in the Labrador sample decreased at day 7 post-confluence. TNAP is a key enzyme involved in mineralization and we are now optimising culture conditions to stimulate matrix mineralisation activity.

Age is an important factor in skeletal maturation/senescence: growth plates in dogs close at 12–18 months old and seniority is reached at 5–13 years depending on dog size and breed. These data provide an important step towards cell culture optimization for canine osteoblasts highlighting the importance of obtaining skeletally mature samples in comparative biology studies.

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P167

Implication of autophagy in a preclinical mouse model of bone ageing and of osteoporosis

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Although estrogen deficiency has been considered for a long time as the main factor leading to osteoporosis (OP), several lines of evidence highlight the role of oxidative stress increase with age as a key factor in this pathology. Autophagy acts as a central mechanism allowing damaged macromolecules and organelles to be degraded and recycled, such as mitochondria, the main source of reactive oxygen species (ROS). Recent publications have shown that autophagy is a new actor in OB function, specifically through its effect on lowering oxidative stress. In parallel, a decrease of autophagy with age has been reported in several cell types. Thus, we could hypothesize that an age related autophagy deficiency in OB could contribute to skeletal ageing in osteoporosis onset. In the present work, we have analyzed autophagy activity in osteocytes (OST) and OB in male and female mice according to their age and hormonal status.

In OST, we observed a decrease in autophagy with ageing while it increases following gonadectomy in males and females. However, although a 70% decrease in autophagy is observed in OB derived from ageing females, this activity remains unchanged in males. While ovariectomy has no effect on OB autophagy levels, orchidectomy leads to a four-fold increase in these cells. These results have been obtained by measuring the LC3-II protein levels in western blots and confirmed by transmission electron microscopy analysis of the autophagic vacuoles. Moreover, we observed an inverse correlation between autophagy and the oxidative stress level in OB derived from males or females.

Thus, our results suggest that OB autophagy levels fluctuate with age and hormonal status in a different way depending on the gender. In females, the lowering of autophagy in OB, which is associated with an increased oxidative stress, could play a role in the construction-resorption balance disequilibrium observed in osteoporosis.

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P168

Two different domains of fibronectin stimulate osteoblast differentiation by activating distinct integrins

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Fibronectin is produced in different isoforms by osteoblasts during differentiation. In contrast to the circulating Isoform, which lacks extra domains, the fibronectin laid down by the osteoblasts in the matrix contains one or both extra-domains called EDA and EDB. We had found that deletion of fibronectin in osteoblasts decreased BMD. This is mediated by diminished osteoblast differentiation and cannot be corrected by the circulating isoform of fibronectin, suggesting a role for EDA and/or EDB in modulating osteoblast behavior.

To evaluate the role of EDA in osteoblasts, wildtype newborn murine calvarial osteoblasts were transiently transfected with a fibronectin construct containing EDA. This enhanced osteoblast differentiation (von Kossa staining, alkaline phosphatase and osteocalcin). In contrast, siRNA directed against EDA suppressed differentiation. This effect was mediated via rac1 and enhancement of wnt signaling. Because EDA increased integrin-mediated signaling as evidenced by increased pFAK and can affect α4β1, α9β1or α5β1 integrins, we deleted β1-integrin in osteoblasts. This prevented EDA-mediated enhancement of differentiation. Inhibiting α4β1 diminished pFAK and osteoblast differentiation. Finally, a peptide called CS1 that activates α4β1 increased total BMD in treated mice.

Similar studies were performed for EDB. EDB enhanced differentiation and its silencing using siRNA inhibited differentiation. However, EDB effects are mediated by β3 integrin, because the absence of β3 prevented EDB effects. Adding echistatin activated β3 in osteoblasts and enhanced differentiation. This was mediated by the RGD sequence because transfecting EDB-fibronectin lacking RGD prevented stimulation by EDB. Furthermore, knockout of β3 in one allele is associated with a decrease in BMD in 5-week-old β3 +/− heterozygote mice, while homozygote loss is not, presumably because β3-integrin is critical for osteoclastic resorption.

In summary, EDA activates α4β1 integrin, while EDB activates β3 integrin. The presence of either domain enhanced osteoblast differentiation. Thus we have clarified the role of two isoforms of fibronectin in osteoblasts.

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P169

Iron deficiency increases osteoblast function via Wnt5a

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Iron overload due to hemochromatosis or chronic blood transfusions has been implicated in the development of osteoporosis. However, the impact of iron overload or iron deficiency on stromal cell functions and the underlying

mechanisms are poorly defined. Since the Wnt signaling pathway is a critical regulator of bone remodelling, we aimed to analyse the effects of iron overload and iron deficiency on osteoblast function and further define the role of Wnt signaling in these processes.
Therefore mesenchymal stromal cells were isolated from the bone marrow of wild type mice and differentiated towards osteoblasts. Treatment of the cells with iron (FeCl3) significantly and dose-dependently attenuated osteoblast differentiation in terms of mineralization and osteogenic gene expression whereas iron chelation with deferoxamine (DFO) promoted osteogenic differentiation of the mesenchymal cells in a concentration-dependent manner.
To elucidate the impact of Wnt signaling in iron-chelation osteogenic promoting effects we performed a Wnt signaling array of DFO-treated osteoblasts. Whereas some Wnt inhibitors such as Sfrp1 and Sfrp2 were significant downregulated, the osteoblast key regulator Wnt5a was increased 3- to 5-fold upon DFO treatment in a time- and dose-dependent manner. Further downstream signaling pathway analysis by applying specific pathway inhibitors revealed that DFO promotes Wnt5a-dependent osteogenic differentiation mainly via PI3K signaling pathway. Finally we could confirm the indispensability of Wnt5a in the DFO-mediated osteoblast promoting effects by analyzing osteoblast differentiation and matrix mineralization of Wnt5a-deficient mesenchymal stromal cells. The DFO-promoting effect on matrix mineralization in wild type cells was completely abolished in $Wnt5a^{-/-}$ cells.
Thus, these data demonstrate that Wnt5a is a target of DFO and a key mediator of the pro-osteogenic effects of iron chelation via DFO.

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P170

Matrix vesicle-mediated mineralization depends on the balance between annexins and fetuin-A which may be modulated by TNAP and calcium channel inhibitors

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Bone mineralization is initiated by matrix vesicles (MVs), cell-derived structures released into the extracellular matrix which are nucleation sites for hydroxyapatite (HA) formation. It is suggested that annexins are mineralization-stimulating membrane proteins that exhibit ion channel activity and facilitate the influx of Ca^{2+} into MVs. The process is also regulated via enzymatic degradation of inhibitory pyrophosphate by tissue-nonspecific alkaline phosphatase (TNAP). Another layer of control is exerted by circulating, mineralization-inhibiting protein fetuin-A.
The objective of our study was to examine the roles of annexins and fetuin-A in MVs function during physiological and pathological mineralization of bones.
We used two human cell lines: osteoblastic hFOB1.19 and osteosarcoma Saos-2. These cells were stimulated for mineralization for 7 and 14 days by ascorbic acid and β-glycerophosphate treatment. We compared cell morphology, intracellular distribution of proteins and formation of HA in control and levamisole (TNAP inhibitor)- or K-201 (a calcium channel inhibitor)-treated cells. We detected calcium nodules by Alizarin Red-S staining of cell cultures. Levamisole blocked mineralization of hFOB and Saos-2 cells. On the other hand, K-201 did not have a significant effect on hFOB cells whereas stimulated calcium deposition in Saos-2 cells. We then isolated MVs from these cells by collagenase digestion and determined TNAP activity using pNPP as a substrate. TNAP activity in osteosarcoma cells was at least 30 times higher than in hFOB cells and reached the maximum after 25 min. Using WB method we observed differences in annexins and fetuin-A profile in MVs from resting vs stimulated cells, but the expression of annexins and fetuin-A was similar in both cell lines. Levamisole decreased the level of fetuin-A in Saos-2 cells, but did not influence the annexin level, whereas K-201 decreased the content of fetuin-A and annexins in both cell lines.
In conclusion, understanding of the role of annexins and fetuin-A as biomarkers in TNAP-regulated function of MVs may provide novel insights into the mechanisms of physiological mineralization and may help to create TNAP and calcium channels inhibitors as therapeutic strategies to prevent pathological mineralization.

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Cell biology: osteoclasts and bone resorption

P171

Notch 2 signaling promotes osteoclast resorption via activation of PYK2

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Notch signaling plays a central role in various cell fate decisions, including skeletal development. Recently, Notch signaling was implicated in osteoclast differentiation and maturation, including the resorption activity of osteoclasts. However, the specific involvement of notch signaling in resorption activity was not fully investigated. Here, we investigated the roles of Notch signaling in the resorption activity of osteoclasts by use of the γ-secretase inhibitor dibenzazepine (DBZ). Attenuating Notch signaling by DBZ suppressed the expression of NFATc1, a master transcription factor for osteoclast differentiation. However, overexpression of a constitutively active form of NFATc1 did not fully rescue the effects of DBZ. DBZ suppressed the autophosphorylation of PYK2, which is essential for the formation of the podosome belt and sealing zone, with reduced c-Src/PYK2 interaction. We further observed increased PYK2 activation by RANKL accompanied by increased NICD2 production. These results confirmed that overexpression of NICD2 rescued DBZ-mediated suppression of resorption activity with promotion of PYK2 autophosphorylation, PYK2/c-Src interaction, and microtubule acetylation. Consistent with the *in vitro* results, DBZ strongly suppressed bone destruction in an interleukin-1-induced bone loss model. Collectively, these results demonstrate that Notch 2 in osteoclasts plays a role in the control of resorption activity via the PYK2-c-Src-microtubule signaling pathway.

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P172

Rooibos (*Aspalathus Linearis*) tea extract inhibits osteoclast formation and bone resorption in RAW264.7 murine macrophages, *in vitro*

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Rooibos (*Aspalathus linearis*) tea is a refreshing and caffeine-free tea indigenous to the Western Cape regions of South Africa. This tea, which is rich in polyphenols and antioxidants, has shown many beneficial effects in health, however, its anti-osteoclastogenic potential remains unexplored. In this study, the *in vitro* effects of an aqueous extract of fermented rooibos tea were examined on osteoclast formation and bone resorption in RAW264.7 murine macrophages. RAW264.7 macrophages were seeded at 15,000 cells/cm^2 in the presence of sterile distilled water (vehicle control) or tea extract (62.5–500 μg/ml). Cell viability was determined by alamar blue assay after 48 h exposure. Osteoclastogenesis was stimulated by the addition of RANKL (15 ng/ml) and evaluated after 5 days by staining for the enzyme tartrate-resistant acid phosphatase (TRAP) and quantification of TRAP-positive multinucleated cells. Actin ring formation was determined by fluorescent stain using a phalloidin conjugate. Bone resorption assays were conducted on osteoassay plates coated with inorganic synthetic bone surface after washing off the cells to visualize resorption pits. The expression of key osteoclast markers was determined via real-time PCR. NF-κB activation was determined by secreted alkaline phosphatase (SEAP) assay after stably transfecting cells with a NF-κB inducible SEAP reporter plasmid. Three independent experiments were conducted in triplicate for each test. We found that rooibos tea extract at 250–500 μg/ml significantly ($P<0.05$) inhibited osteoclast formation and TRAP activity, which was accompanied by reduced bone resorption and disruption of characteristic cytoskeletal elements of mature osteoclasts, without cytotoxicity. Rooibos tea extract decreased expression of key osteoclast specific genes, matrix metalloproteinase-9, TRAP and cathepsin K. Furthermore, the tea extract inhibited the activation of the intracellular signalling marker, NF-κB, at increasing concentrations after 48 h. This study demonstrates for the first time that rooibos tea may have potential anti-osteoclastogenic effects.

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P173

Bone resorption by *Tannerella forsythia* GroEL

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Tannerella forsythia is a gram-negative, anaerobic, asaccharolytic, fusiform bacterium. The presence of the bacterium is associated with various forms of periodontal disease, including gingivitis, chronic and aggressive periodontitis. The expression of GroEL, a bacterial heat shock protein, increases in stressful conditions such as infection. GroEL is a moonlighting protein that has multiple functions. The aim of this study was to evaluate the effect of *T. forsythia* GroEL alone or GroEL in the presence of IL-17 on bone resorption. BALB/c mice were injected with *T. forsythia* GroEL (20–50 μg/mouse) and/or IL-17 (1 μg/mouse) subcutaneously over the calvaria daily for 5 days. On day 7 after the first administration, mice were sacrificed and bone resorption in the calvaria was analyzed by micro-CT and TRAP staining. *T. forsythia* GroEL increased the area of bone resorbed and the size and the number of the resorption spots. IL-17 synergistically enhanced GroEL-induced bone resorption. These results suggest that *T. forsythia* GroEL alone or in the presence of IL-17 may play an important role in modulating bone resorption which is a characteristic of periodontitis.

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P174

Abstract withdrawn.

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P175

A jumonji histone demethylase inhibits osteoclast differentiation through NFATc1 regulation

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Osteoclasts are bone-resorbing multinucleated cells that differentiate from monocyte/macrophage-lineage precursors. Bone destruction and osteoporosis are attributed to excessively activated osteoclasts. Osteoclast differentiation is governed by diverse regulatory processes including nuclear factor-activated T cells c1 (NFATc1) activation in response to RANKL. The mechanism of epigenetic regulation of NFATc1 in osteoclastogenesis not investigated yet. Here we test a hypothesis that a jumonji histone demethylase might epigenetically regulate NFATc1 during osteoclast differentiation. Western blot analysis showed decrease of jumonji histone demethylase expression during osteoclastogenesis of RAW264.7 cell line. The knock down expression of a jumonji histone demethylase in RAW264.7 and bone marrow macrophages facilitated the osteoclastogenesis through RANKL. Luciferase reporter assay showed that the suppression of a jumonji histone demethylase also increased the transcriptional activity of NFATc1. Immunoprecipitation assay showed that a jumonji histone demethylase directly binds to the C-terminal of NFATc1. From the above results, I suggest that a jumonji histone demethylase plays a suppressive role in osteoclastogenesis. As for part of the signaling mechanisms of the inhibition of transcriptional activity, direct protein–protein interaction between a jumonji histone demethylase and NFATc1 was suggested.

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P176

Impaired c-kit signaling couples bone resorption to bone formation through wnt10b in *kit*$^{w\text{-}Sh/W\text{-}Sh}$ mice

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Kit ligand/*c-Kit* receptor tyrosine kinase complex has been implicated as a target for bone remodeling process. Loss of function mutation in *c-Kit* causes low bone mass in $Kit^{W/W\text{-}v}$ (W/W^{v}) mice. However, these mice are sterile and it is unclear whether the observed skeletal phenotype is secondary to sex hormone deficiency. To address this question, the skeletal phenotype of $Kit^{W\text{-}sh/W\text{-}sh}$ (W^{sh}/W^{sh}) mice, which are fertile, was identified. W^{sh}/W^{sh} mice, which carried an inversion mutation affecting the transcriptional regulatory elements upstream of the *c-Kit* promoter region, exhibited osteopenia with elevated bone resorption and bone formation. Histomorphometry indicated an increase in osteoclast number, bone formation rate and mineral apposition rate at 6 and 9 weeks old. *c-Kit* mutation increased osteoclast differentiation. FACS analysis indicated an increase in the percentage of c-Fms^{+}CD11b^{+} cells in spleen of W^{sh}/W^{sh} mice compared to controls. In primary osteoblast culture, W^{sh}/W^{sh} osteoblasts had increased number of committed osteoblast progenitors, alkaline phosphatase and mineralized bone nodules. These changes were associated with increases in steady-state mRNA levels for osteoblast marker genes, including osteocalcin, Osterix, alkaline phosphatase, type I collagen and Runx2, in femurs. *c-Kit* was expressed in both osteoclasts and osteoblasts and mutation of *c-Kit* decreased its expression level in W^{sh}/W^{sh} osteoclasts but not osteoblasts, suggesting an indirect effect of *c-Kit* on bone formation. Osteoclast-derived coupling factor Wnt10b mRNA was increased in W^{sh}/W^{sh} osteoclasts. W^{sh}/W^{sh} osteoclasts produced elevated Wnt10b protein level. Antagonizing Wnt10b with Wnt inhibitor DKK1 reduced W^{sh}/W^{sh} osteoclast conditioned medium-induced alkaline phosphatase activity and mineralization in osteoblast cultures. Our data suggest that *c-Kit* is a negative regulator of bone turnover, and that increased bone formation following disruption of *c-Kit* signaling is driven by osteoclast-derived Wnt 10b.

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P177

A novel regulatory factor in osteoclastogenesis DCL-1/CD302: significance of its binding to CCN2/CTGF

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CCN2/CTGF is known as a multi-functional growth factor for various mesenchymal cells including chondrocytes, osteoblasts, vascular endothelial cells and its function is suggested to be produced by its binding to other growth factors or membrane proteins. Therefore, finding out these binding partners are critically important in understanding the molecular function of the CCN2. As a result of screening, DCL-1/CD302 was found as a new candidate of CCN2-binding molecule. DCL-1/CD302 is one of C-type lectin receptors but the distribution and the function are mostly not clarified. Among a few reports on DCL-1/CD302, there is a report that it is express in macrophages but no report on expression and role of DCL-1/CD302 on pre-osteoclasts or osteoclasts that are the same lineage cells of macrophages. In the present study, we investigated significance of interaction between CCN2 and DCL-1/CD302 in osteoclastogenesis.
Gene expression of DCL-1/CD302 assayed by RT-PCR increased during *in vitro* osteoclastogenesis using mouse* bone marrow cells stimulated by M-CSF and RANKL. Westernblot analysis also confirmed increase in DCL-1/CD302 protein during osteoclastogenesis. Immunoprecipitation and western blotting assay using lysates of osteoclasts derived from muse bone marrow cells and recombinant CCN2 confirmed binding of DCL-1/CD302 to CCN2. Immunostaining revealed that DCL-1/CD302 localized with actin ring (together with CCN2) in mature osteoclasts. Suppression of DCL-1/CD302 by its siRNA inhibited maturation of

osteoclasts and formation of bone resorption pits in vitro. The siRNA targeting DCL-1/CD302 also caused fragmentations of actin ring. Because we previously reported that CCN2 binds to action[1], these data suggest that DCL-1/CD302 has a critical role in actin ring formation and that CCN2 may play an important role in to this process.

Reference

1. Yosimichi G. *et al.* BBRC, 2002 **299** 755–756. *Animal experiments were all approved by the Animal Committee of Okayama University.

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P178

Retrieval of resorptive human osteoclasts from temperature-responsive plastic

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Osteoclasts are the major bone resorbing cells, essential for bone turnover and development. Human osteoclasts can be generated *in vitro* by stimulation of peripheral blood mononuclear cells (PBMCs) with M-CSF and RANK-L. Seeding fully differentiated osteoclasts onto mineralized surfaces facilitates the analysis of molecular interactions between the osteoclast and the mineralized matrix.

Currently, a widely used protocol for harvesting osteoclasts from tissue-culture plastic involves trypsinization and subsequent scraping of the osteoclasts into suspension. Trypsinization and scraping may affect processes such as initial attachment on the bone and initiation of resorption. Therefore, there is a need for an alternative harvesting method. A potential technique involves the use of culture plastics grafted with a temperature-responsive polymer. These culture plastics are cell adhesive at 37 °C. At room temperature (RT), the PIPAAm chains become extended, resulting in the release of adherent cells.

Human PBMCs were differentiated towards osteoclasts on temperature-responsive culture plastic (Thermo Scientific Nunc Up-Cell™). When multi-nucleated osteoclasts had formed, cells were released into suspension by incubation at RT. As a control, osteoclasts were harvested from traditional culture plastic by trypsinization and scraping.

The resulting cell suspensions were reseeded on bovine cortical bone slices. The cells were cultured on bone for a maximum of 48 h. Osteoclasts were detected by staining for TRAcP. Actin rings were visualized by FITC-conjugated phalloidin and the nuclei by DAPI. In order to analyze the osteoclastic resorption activity, cells were removed from the bone slices and resorption pits were revealed by WGA-lectin.

The results showed that multinucleated viable osteoclasts could be harvested from temperature-responsive culture plastic by temperature reduction. The obtained osteoclasts quickly attached to the bone surface and showed formation of actin rings ($<$15 min). Osteoclasts retrieved from temperature-responsive culture plastic showed significantly higher resorption activity compared to osteoclasts harvested by trypsinization and scraping.

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P179

Tensin 3 is a novel partner of dock5 that controls osteoclast podosome organization and activity

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Osteoclasts resorb bone matrix through a specific adhesion structure called the sealing zone or actin ring, which is based on a belt of podosome. Much remains to be uncovered regarding the molecular mechanisms driving podosome organization into superstructures in particular the osteoclast podosome belt. Proteomic analyses in osteoclasts revealed the adaptor protein tensin 3 as a partner of Dock5, a Rac exchange factor necessary for podosome belt formation and bone resorption. Expression of tensin 3 and Dock5 concomitantly increase during osteoclast differentiation. They associate with the osteoclast podosome belt but not with individual podosomes, contrarily to vinculin. Super-resolution microscopy revealed that, even if they colocalize in the x-y plane of the podosome belt, Dock5 and tensin 3 differentially localize relative to vinculin in the z-axis. Tensin 3 increases Dock5 exchange activity towards Rac and suppression of tensin 3 in osteoclasts destabilizes podosome organization, leading to delocalization of Dock5 and severe reduction in osteoclast activity. Our results suggest that Dock5 and tensin 3 cooperate for osteoclasts activity, ensuring correct podosomes organization.

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P180

High trabecular bone mass induced by reduced function of osteoclasts in GULP1-deficient mice

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Engulfment adaptor phosphotyrosine-binding (PTB) domain containing 1 (GULP1) is an adaptor protein involved in the engulfment of apoptotic cells via phagocytosis. Although GULP1 is widely expressed in various tissues, including the brain, muscle, testis, and bone, the function of GULP1 has not been well studied. Here, we investigated whether GULP1 plays a role in the regulation of bone remodeling and examined its expression in bone cells. Conditional *Gulp1* floxed mice were generated by homologous recombination in C57BL/6 embryonic stem cells. Then, *Gulp1* knockout mice were prepared by crossing *Gulp1* heterozygous mice generated by FLP- and Cre-mediated recombination in conditional *Gulp1* floxed mice. The trabecular bone mass of the femur and tibia, as well as the lumbar vertebrae was significantly increased in *Gulp1* knockout mice compared to that their wild-type littermates. The dynamic bone formation of osteoblasts in *Gulp1* knockout mice did not differ from that in wild-type mice. However, the number of fully differentiated osteoclasts and the surface area of the resorption pit in bone slices were lower in *Gulp1* knockout mice than in wild-type mice. Furthermore, actin ring and microtubule formation in osteoclasts were inhibited in *Gulp1* knockout mice. These results indicate that GULP1 deficiency suppresses osteoclast differentiation and bone resorption, but does not alter the bone formation of osteoblasts, which ultimately increases bone mass. Taken together, these results suggest that GULP1 may be a novel positive regulator of osteoclast differentiation and bone resorption.

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P181

Dual-specificity tyrosine-phosphorylation regulated kinase 2 negatively regulates osteoclast fusion

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During the differentiation of the osteoclast, it transform from a mono-nucleated cell to multinucleated cell by fusion of its precursors. Although, cell fusion is a complicated process which likely involves many regulating proteins, only a few osteoclast fusion regulating proteins were identified. We have identified dual-specificity tyrosine-phosphorylation regulated kinase 2 (Dyrk2) as a novel regulator of the osteoclast fusion process. Dyrk2 belongs to a family of protein kinases whose members are involved in cell cycle and cytoskeleton organization. We found that Dyrk2 mRNA expression increases, 48 hours after induction of osteoclast differentiation *in vitro*, a time point were fusion commence. Dyrk2 knock-down, increases osteoclast nuclei numbers, but not osteoclast numbers and area, in differentiating RAW 264.7 cells and primary bone marrow derived monocytes (BMMs), While, overexpression of Dyrk2 decreases the number of osteoclast and osteoclast nuclei numbers suggesting it is involved in regulation of the fusion process per se. In order to further determine if Dyrk2 is involved in the fusion mechanism and not in earlier differentiation steps that lead to a fusion competent state, we investigated its role in giant macrophage fusion which are

induced by different signaling pathways but share a similar fusion mechanism. Knockdown of Dyrk2 in giant macrophages increased the number of nuclei per macrophage, supporting a more general role of Dyrk2 in limiting the fusion process. While attempting to uncover the mechanism at which Dyrk2 regulates fusion we found that the rate of fusion of multinuclear cells is faster in Dyrk2 deficient osteoclasts. Moreover an ectopic expression of a GFP-tagged Dyrk2 showed co localization of Dyrk2 and actin in osteoclasts actin rings known to regulate osteoclast fusion. Taken together, our data reveal a novel role for Dyrk2 as a negative regulator of osteoclasts and giant macrophage fusion likely through the modulation of the osteoclast cytoskeleton.

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P182

Human dendritic cell-derived osteoclasts have the ability for both bone absorption and T cell stimulation

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Objective
It is well known that monocytes differentiate into osteoclasts. However, we found that human dendritic cells (DCs) also differentiate to osteoclast-like cells and assessed the role of dendritic cell-derived osteoclast (DC-OC) in pathological processes of inflammatory diseases such as rheumatoid arthritis (RA).
Methods
DC-OCs were differentiated from human monocyte-derived DCs *in vitro*. The function of DC-OCs was compared to human monocyte-derived osteoclasts (Mo-OCs). Tartrate-resistant acid phosphatase (TRAP) and immunohistochemistry staining were used to detect osteoclasts and DC-OCs in RA synovium.
Results
The culture of human DCs with M-CSF and RANKL *in vitro* resulted in the differentiation into multi-nucleated DC-OCs, which were positive for OC markers such as TRAP and cathepsin K. These DC-OCs had the strong bone absorption capacity by the Pit-formation assay. On the other hand, DC-OCs expressed major histocompatibility complex-class II and costimulatory molecules such as CD80 and CD86, which were not accompanied in Mo-OCs. Furthermore, co-culturing of DC-OCs with $CD4^+$T cells increased T cell proliferation, whereas Mo-OCs did not, indicating that DC-OCs had antigen-presenting activities. Finally, TRAP-positive multinucleated OCs were detected in RA synovium. Of note, 33% of these cells beard CD86.
Conclusion
This is the first report showing that human DCs can differentiate into DC-OCs in the inflammatory lesion without being regulated by osteoblasts/osteocytes. DC-OCs possess not only the bone resorption ability but also the antigen-presenting function as DC. Actually, there were characteristic osteoclasts bearing costimulatory molecules in RA synovium, indicating that DC-OCs may play a pivotal role in the pathogenesis of RA by the maintenance of inflammation as well as joint destruction.

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P183

Life span differs between osteoclasts derived from different bone marrow precursors: a time-lapse microscopy study

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Osteoclasts are multinucleated bone-resorbing cells and can be cultured from different monocytic precursors. It is unknown whether osteoclasts derived from different precursors are phenotypically different. One of the aspects not known is the life span of the different osteoclasts and the effect of IL-1β hereupon. Here, we studied this using time-lapse microscopy. Bone marrow cells were isolated from 6-week-old male mice. Early blasts ($CD31^{hi}Ly\text{-}6C^-$), myeloid blasts ($CD31^+Ly\text{-}6C^+$) and monocytes ($CD31^-Ly\text{-}6C^{hi}$) were sorted using flow cytometry. Cells were cultured in the presence of M-CSF and RANKL, with or without 10 ng/ml IL-1β on plastic and visualized by time-lapse microscopy. The stimulatory effect on multinucleation by IL-1β, as shown previously by others, was confirmed. We found that large osteoclasts (>10 nuclei) generated from the three subsets in the presence of IL-1β differed remarkably in their life span. The myeloid blast-derived large osteoclasts were found earliest, after 75 h; these cells survived for only 30 h. The monocyte-derived large osteoclasts were found 20 h later, after 95 h. These cells, however, survived the longest, being 50 h. The early blast-derived-large osteoclasts were in between, appeared after 85 h with life span of 40 h. Next we analyzed the life span of individual osteoclasts and found that osteoclasts that became large (>10 nuclei) had a significantly shorter life span than osteoclasts that remained small (<10 nuclei), being 13 ± 7 h for large osteoclasts and 51 ± 16 h for the smaller ones. The large osteoclasts generated by monocytes in the presence of IL-1β had a significantly longer life span (22 ± 8 hours/osteoclast) than the osteoclasts generated by early blasts (12 ± 4 hours/osteoclast) and myeloid blasts (12 ± 3 hours/osteoclast). In conclusion, considerable differences occur between the life span of osteoclasts derived from different bone marrow precursors. We propose that depending on the inflammatory situation, osteoclasts with various life span can be generated.

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P184

Involvement of integrin beta 2/CD18 in attachment of monocytes to bone

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Background
Osteoclasts (OCs) are bone-degrading cells that differentiate from the monocyte/macrophage lineage. In human, three monocyte subsets have been identified: classical, intermediate and non-classical monocytes. We have previously demonstrated that comparable numbers of OCs can be generated from these subsets on plastic, but that the number of OCs significantly differs when the monocytes are cultured on bone. It is plausible that the observed differences are associated with initial attachment of the OC precursors to bone. However, the mechanism involved in attachment of OC precursors to bone is unknown.
Objective
To evaluate attachment of the three monocyte subsets to bone and their early differentiation towards OCs.
Methods
Monocytes were isolated from human peripheral blood and sorted with flow cytometry based on CD14 and CD16 expression. The subsets were seeded onto slices of human tibia bone. Differentiation was induced by osteoclastogenic medium containing 10 ng/ml M-CSF and 2 ng/ml RANKL. At baseline, and after 3, 18, 24 and 48 h of culture, monocyte attachment was visualized by staining the nuclei and surface expression of integrins β2, αM and αL was analysed with flow cytometry and confocal microscopy.
Results
Attachment to bone and early osteoclastogenesis of the three monocyte subsets was distinctly different. Classical monocytes attached readily and formed cell clusters shortly after seeding. Intermediate and non-classical monocytes attached less well to bone and no clusters were formed. Classical monocytes expressed high levels of integrin β2 and blocking of plasma membrane associated integrin β2 effectively inhibited monocyte attachment to bone.
Conclusion
Integrin β2 appears to be involved in the adhesion of monocytes to bone.
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P185

Characterising the role of the lysosomal membrane proteins MFSD1 and TMEM106b in osteoclasts

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Osteoclasts are highly specialized cell types, responsible for the resorption of bone matrix. Coordinated with osteoblasts they contribute to a proper bone turnover. An impaired or reduced function of the osteoclast leads to a pathogenic increase of the bone mass and finally osteopetrosis. Lysosomal hydrolases, as exemplied by CTSK and Acp5 (TRAP), are known to play an important role in the function of osteoclasts, and knockout mouse models of these proteins develop a bone resorption phenotype. Apart from these soluble lysosomal enzymes, the importance of lysosomal membrane proteins in the osteoclast resorptive function such as CLC-7, OSTMN1 and ATP6a3 is characterized by the regulation of the conditions in the resorption lacuna. Mutations or knockout of these genes in human patients and mouse models, respectively, also lead to ostepetrotic phenotypes.

Due to the similarities between the ruffled border and the limiting membrane of the lysosome, it is of great interest to generally address the possible role of lysosomal membrane proteins in osteoclasts. MFSD1 and TMEM106b are two recently identified lysosomal membrane proteins, whose function has not been yet unravelled. Mfsd1 is a lysosomal transporter for so far unknown metabolite(s). The function of Tmem106b in osteoclasts is also unknown, but it possibly mediates the retrograde transport of lysosomes in neuronal dendrites.

MFSD1 is highly present in bone marrow macrophages, and its expression is upregulated upon stimulation with MCSF and RANKL, reaching its maximum after 3 days of treatment. MFSD1 is an unglycosylated protein. However, subcellular localization by immunofluorescence revealed the presence of MFSD1 in lysosomes in differentiated osteoclasts.

Analysis of osteoclasts differentiation from bone marrow and its capacity of resorption, using the KO mouse model for MFSD1 and TMEM106b will allow us to get a deeper insight of the importance of both proteins in osteoclast biology.

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P186

Inflammatory conditions induces a new subset of osteoclasts that prime TNFα-producing CD4+T cells

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Chronic inflammatory diseases are characterized by a bone destruction mediated by an increased osteoclast (OCL) activity. OCLs are phagocytic cells arising from the myeloid lineage. Indeed, OCLs derive from monocytes (MN-OCLs) and, in an inflammatory context, they also derive from dendritic cells (DC-OCLs). Despite this origin, their role in the immune responses is still unclear. OCLs in steady state have been reported to act as antigen-presenting cells that activate CD8+ regulatory T cells, revealing an immune suppressive function, but such function has never been studied in an inflammatory context.

Our aim was to address the effect of OCLs from different origin on CD4+ T cell responses. We set up a unique procedure to purify OCLs on a cell sorter to analyze OCL specific immune function. Working with pure OCL populations, we showed that MN-OCLs and DC-OCLs have the same capacity to process and present antigens. On the other hand, DC-OCLs express high levels of inflammatory cytokines; they efficiently attract CD4+ cells, and induce their differentiation into TNFα-producing T cells. In contrast, MN-OCLs are not efficient in attracting CD4+ T cells; they induce their differentiation into regulatory T (Treg) cells and express higher levels of immunosuppressive IL-10. These results were confirmed using a murine model of colitis associated with an overactivation of OCLs, the $Rag1^{-/-}$ mice transferred with naive CD4+ T cells. As MN-OCLs, OCLs from control mice induce CD4+ T-reg cells, whereas those from colitic mice have the same inflammatory properties than DC-OCLs. Our results demonstrate that MN-OCLs are related with the BM tolerance in steady state, probably avoiding self-reactivity against the peptides continuously produced during bone resorption. In contrast, under inflammatory conditions DC-OCLs may induce inflammatory or autoimmune responses and participate to an amplification loop between bone destruction and inflammation.

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P187

Identification of CX3CR1 as the first known marker of inflammatory osteoclasts

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Existence of inflammatory osteoclasts (iOCLs) contributing to pathological bone resorption associated with inflammatory diseases has been suspected for many years. However, specific markers of iOCLs are lacking, making impossible to establish the contribution of iOCLs to such pathologies. Whereas in steady state OCLs derive from monocytes (MN-OCLs), in inflammatory conditions, they also differentiate from dendritic cells (DC-OCLs). We recently showed that these 2 OCL populations are antigen-presenting cells that drive different CD4+ T cells responses in vitro. MN-OCLs induce the differentiation of CD4+ T cells into regulatory T (Treg) cells and DC-OCLs induce their differentiation into TNFα-producing cells. These differences were confirmed with OCLs generated from normal or inflamed mice using a murine model of inflammatory bowel disease (IBD) associated with severe bone destruction, the $Rag1^{-/-}$ mice transferred with naive CD4+ T cells. Contrasting with OCLs from control mice that induce CD4+ Treg cells as MN-OCLs, OCLs from IBD mice have inflammatory properties similar to DC-OCLs. Taking advantage of these different tolerogenic or inflammatory OCL populations, our aim was to identify cell surface markers to characterize iOCLs subsets. We have performed a comparative flow cytometry analysis of DC-OCLs and MN-OCLs on about 20 surface markers described for monocytes and dendritic cells. We have observed that expression of CX3CR1, the receptor of fractalkine, is restricted to DC-OCLs. Using the IBD model, we have confirmed that CX3CR1+OCLs are characteristic of IBD development. Moreover, these CX3CR1+OCLs induce inflammatory CD4+ T cells but not Treg cells. Lastly, we have shown that the emergence of CX3CR1+ OCLs is controlled by IL17. These results are the first characterization of a marker allowing the identification of iOCLs. They provide also an essential tool for the study, diagnosis and therapeutic targeting of i-OCLs.

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P188

The Arf and Rho GTPase-activating protein (GAP) ARAP1 regulates osteoclast function

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The ArfGAP with RhoGAP domain, ankyrin repeat and PH domain containing protein 1 (ARAP1) is a multi-domain protein that binds to phosphatidylinositol lipids within different biological membranes and integrates Rho and Arf signalling pathways. It is ubiquitously expressed in many cells including osteoclasts and its overexpression is known to affect Golgi and induce filopodia by activation of Cdc42. We therefore hypothesized that ARAP1 might also play an important role in osteoclasts by controlling both osteoclast polarity and post-Golgi transport to the ruffled border, two key events necessary for bone degradation.

We found ARAP1 at two different localizations in osteoclasts, on actin-rich podosomes that condense to sealing zones and at vesicles in the peri-nuclear region, rich in Lamp1, actin and the adaptor protein complex 3 (AP-3). This is in agreement with previous studies where ARAP1 was found in the network of AP-3 vesicles. AP-3 is responsible for targeting selected transmembrane proteins as LAMP1 to lysosomes and lysosome-related organelles; its role in vesicular transport to the ruffled border however remains elusive. Live-cell imagining revealed that ARAP1 dynamics follow those of actin at podosomes and sealing zones. Osteoclasts with overexpressed ARAP1 were also AP-3 positive but remarkably those compartments were enlarged, pointing to a key role of ARAP1 in AP-3 endosome maturation.

Knockdown of ARAP1 results in the disruption of the podosomal belt organization, changes in the classical osteoclast cell morphology and strikingly significant reduction in resorption capacity.

In addition knockdown of the AP3-μ1 subunit, which results in the dissociation of the AP-3 complex, leads to significantly increased resorption of targeted osteoclasts, possibly by disrupting AP-3-dependent lysosomal membrane protein trafficking to the ruffled border. Based on this data we suggest that ARAP-1 is important for osteoclast function but also for AP-3 dependent trafficking, which appears to be a key machinery in osteoclast activity.

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P189

Pathophysiological implication of Autotaxin on osteoclast function

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Autotaxin (ATX) is a secreted protein produced by various tissues in the body including the liver, adipose tissue and bone. Autotaxin (ATX) is an enzyme with a phospholipase D activity responsible for cleavage of lysophosphatidyl-choline (LPC) in lysophosphatidic acid (LPA). LPA is a bio phospholipid, which acts as a growth factor, affecting proliferation, differentiation, and migration. It has been shown that the biological effect of LPA could be the direct consequence of local production of Autotaxin (ATX) in a given tissue or cell type[1]. Recently, we have shown that LPA controls two steps of osteoclastogenesis: the fusion and bone resorption capacity of osteoclasts[2]. The aim of this study is to test if ATX produced by osteoclasts could play a direct role on osteoclastogenesis and in bone mass control. First we observed that *Enpp2* (ATX gene) was a target gene of RANK-L and as a consequence was up regulated during the course of osteoclastogenesis from bone marrow (BM) wild type (WT) cells. Next we generated Ctsk-Cre+; ATXfl/fl mice and use of these animals as a source of osteoclasts-ATX deficient progenitors. *In vitro* experiments showed a major impact of ATX on osteoclastogenesis and osteoclast mediated bone resorption. Because LPA is massively present in sera, to explore either LPA or ATX putative effect in culture, the use of delipidated serum was mandatory. Using such conditions, we observed a drastic reduction in the number of mature osteoclasts after 5 days of differentiation from BM-WT progenitors, but osteoclasts number were restored by the use of LPA, recombinant ATX plus LPC, or LPC. These results were confirmed by the use of LPA and ATX specific inhibitors (KI16425 and PF8380). Osteocorning bone resorption assays showed that ATX is required for osteoclasts activity, that decreased of 50% when delipidated sera is used in the assays and that was fully restored by the addition of either LPC or recombinant ATX plus LPC. All together, and more specifically the results obtained in presence of LPC alone suggest that i) ATX is secreted by osteoclasts and ii) is functionally involved in osteoclast differentiation and function *in vitro*. Current studies are conducted on Ctsk-Cre+; ATXfl/fl mice to fully characterize the role of ATX in the bone mass control in physiological and pathological conditions due to ageing (osteoporosis) and inflammation (rumathoid arthritis).

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P190

Identification and characterisation of vesicles in resorbing osteoclasts using electron tomography

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Osteoclasts are the only cell type capable of resorption of mineralised matrix such as bone or dentine. Resorbing osteoclasts form distinct membrane domains: the functional secretory, the basolateral and the ruffled border (RB) domains. The RB allows acidification of the resorption lacuna, exocytosis of osteolytic enzymes and uptake of degraded bone material, processes that require directed vesicular transport. Few studies have tried to classify the vesicles near the RB into secretory or uptake pathways and given their size (100–500 nm) such studies are not possible by light microscopy alone. Using electron tomography, we characterised vesicles adjacent to the RB. Rabbit osteoclasts resorbing dentine discs were processed for transmission electron microscopy (TEM) using standard fixation and embedding techniques. 200 nm thick sections were imaged in a JEOL JEM-1400Plus TEM. Tilt series (±60 degrees) around the structure of interest were acquired with a 2D image captured at each degree. 3D tomograms were generated and regions of interest rendered using Amira software. These renderings allowed better interpretation of 2D TEM images of osteoclastic vesicles and some new observations were made. i) Single membrane-bound vesicles with electron dense content and distinct halos, previously thought to be secretory lysosomes, were found to be tangential sections of collagen fibrils encased in RB membrane. ii) Single membrane-bound vesicles with moderate electron dense content located near the RB were often associated with extracellular collagen fibril tips. They appeared to contain small amounts of degraded collagen and therefore may be part of the uptake pathway. iii) Large, double membrane-bound vesicles (autophagosomes) were frequently seen near the ruffled border. iv) Vesicles (0.1–1.5 μm) without notable content were released from the apical membrane. Our observations illustrate the need for 3D imaging to avoid erroneous interpretation of 2D TEM and begin to describe the vesicles involved in osteoclastic resorption.

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P191

Homer proteins modulate RANKL-induced NFATc1 signaling in osteoclast differentiation and bone metabolism

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Ca^{2+} signaling and NFATc1 activation are essential for RANKL-induced osteoclast differentiation through the induction of Ca^{2+} oscillation, calcineurin activation, and translocation of NFATc1 into the nucleus. Homer proteins are scaffold proteins that have been proposed to modulate multiple Ca^{2+} signaling proteins, including inositol 1,4,5-triphosphate receptors, ryanodine receptors, transient receptor potential channels. In this study, we investigated the role of Homer2 and Homer3 in Ca^{2+} signaling during osteoclast differentiation using Homer2/Homer3 (Homer2/3) double-knockout (DKO) mice. Deletion of Homer2/3 markedly decreased the bone density of the tibias, resulting in bone erosion. Homer2/3 DKO bone marrow-derived monocytes/macrophages (BMMs) facilitated greatly osteoclast differentiation through increased NFATc1 expression and translocation of NFATc1 into the nucleus after 48 h of RANKL treatment. Notably, the interaction of Homer proteins with NFATc1 was inhibited by RANKL treatment, but restored by cyclosporine A treatment to inhibit calcineurin. Finally, RANKL treatment of Homer2/3 DKO BMMs significantly increased approximately threefold induction of multinucleated cells formation. These findings suggest that Homer2/3 regulate NFATc1 function by interacting with NFATc1 it in the cytosol and thus modulate the NFATc1 pathway and RANKL-induced osteoclast differentiation and bone metabolism. This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIP) (2015R1A2A1A15054157) and (MOE) (2015R1D1A1A01057277).

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P192

Regulation and function of lentiviral-mediated TCIRG1 expression in osteoclasts from infantile malignant osteopetrosis patients

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Infantile malignant osteopetrosis (IMO) is a rare, lethal, recessive disorder characterized by dysfunctional osteoclasts. TCIRG1, encoding the osteoclast V-ATPase, is mutated in 50% of IMO patients. We have previously shown that the resorptive function in osteoclasts derived from IMO patients can be restored *in vitro* by expressing TCIRG1 using a lentiviral vector. In this study, we aim to investigate the cellular response to vector-derived TCIRG1 expression and to determine the TCIRG1 expression needed to restore resorptive function.

$CD34^+$ cells from peripheral blood of IMO patients were transduced with a lentiviral vector expressing TCIRG1 and GFP under the SFFV promoter. The cells were expanded for 2 weeks followed by *in vitro* differentiation towards osteoclasts or macrophages for 3, 5, 9 or 14 days. TCIRG1 expression was analyzed by western blot and was only observed in mature osteoclasts in contrast to GFP which was observed under all conditions.

The threshold needed to restore resorptive function *in vitro* was assessed by mixing $CD34^+$ cord blood (CB) cells or transduced $CD34^+$ IMO cells with untreated $CD34^+$ IMO cells and differentiating them into osteoclasts on bone slices. TRAP activity and CTX-I release were measured in the media. Mixing 30% CB cells with IMO cells was sufficient to completely normalize resorptive function measured by resorption per osteoclast (CTX-I/TRAP). Doses as low as 2.5% transduced IMO cells mixed with untreated IMO cells were capable of increasing the resorption per osteoclast reaching up to a 14-fold increase at 30% transduced IMO cells.

In conclusion we show that lentiviral-mediated expression of TCIRG1 is regulated in the same manner as endogenous TCIRG1 despite being expressed by a constitutively active promoter. This suggests that TCIRG1 is post-

transcriptionally regulated. We furthermore show that only a low fraction of human pre-osteoclasts with functional TCIRG1 is needed to significantly increase resorptive function *in vitro*.

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P193

C-C chemokine receptor 5, a co-receptor of HIV, -mediated signal is required for geometric architecture and function of osteoclasts, thus for RANKL-induced bone destruction

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C-C chemokine receptor 5 (CCR5) is a co-receptor of macrophage-tropic viruses including HIV. Epidemiological and pathological findings have reported that functional changes in CCR5 correlate with HIV transmission bone destruction disease. However, the roles of CCR5 in bone pathophysiology have not been well documented.

Ccr5-deficient osteoclasts showed decreased bone resorption activity accompanied with disorganized cellular architecture and impaired motility. Multimodal and multidimensional super-resolution microscopy facilitates to observe irregular microtubule network and podosome arrangement in $Ccr5^{-/-}$ osteoclasts, suggesting malfunctions for cell polarity, cell adhesion and locomotion. Time-lapse imaging and subsequent numerical analysis of cell deformity index revealed that *Ccr5*-deficient osteoclasts reduced their stability of attachment to matrix as evidence by abnormal motility. Expression of integrins and signaling of FAK-Src complex were markedly reduced in Ccr5-deficient osteoclasts, which was concomitant to reduced activity of small GTPase. CCL5, a major ligand of CCR5, stimulated FAK phosphorylation not Src in wild type osteoclasts. RANKL-induced Src phosphorylation and downstream signals were enhanced by CCL5 stimuli, suggesting a cooperative role of CCR5-mediated signaling with RANKL-mediated signaling pathways. Forced expression of constitutive active forms of Rho and Rac in *Ccr5*-deficient osteoclasts rescued integrin expression and bone resorption. These findings suggested that CCR5-mediated signaling, with cooperating with RANKL-mediated signaling, regulate small GTPases, and thus cellular architecture and motility of differentiated osteoclasts.

Ccr5-deficient bone had significantly increased osteoclasts number, although they did not show difference in BMD compared to their wild-type littermates, indicating dysfunction of *Ccr5*-deficient osteoclasts *in vivo*. Interestingly, *Ccr5*-deficient osteoclasts were observed to be flattened in shape with covering wider bone surface compared to those in wild type in bone sections, supporting our *in vitro* findings. Furthermore, *Ccr5*-deficient mice were less susceptible to RANKL-induced bone loss model.

Our findings suggested critical role of CCR5-mediated signaling in pathological bone destruction, thus its implications of bone-specific therapeutic targets.

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P194

Neuropetide Y Y_1 receptor deletion impairs matrix demineralization and resorption

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Neuropeptide Y Y_1 receptor (Y_1R) signalling has been shown to play a key role in bone homeostasis, emerging as a novel therapeutic target in bone diseases. Y_1R knockout mice ($Y_1^{-/-}$) display a high-bone mass phenotype that has been mainly attributed to increased osteoblast activity. Nevertheless, the Y_1R regulatory role on osteoclastogenesis and matrix resorption remains largely unknown. To clarify this, we proposed to investigate the effects of Y_1R in osteoclast function and matrix demineralization/resorption, using bone marrow-derived osteoclasts retrieved from $Y_1^{-/-}$ mice and compared to their wild-type (WT) counterparts ($n=6$ per genotype).

The number of TRAP-positive multinucleated cells was significantly elevated in $Y_1^{-/-}$ cultures, when compared to WT ($P<0.001$). Moreover, $Y_1^{-/-}$ osteoclasts surface area and number of nuclei ($N>8$) were also significantly increased ($P<0.01$), suggesting enhanced formation and osteoclast fusion, possibly due to an overexpression of monocyte chemoattractant protein-1 (MCP-1) in $Y_1^{-/-}$ cultures ($P<0.01$). Moreover, osteoblast/osteoclast direct co-cultures demonstrated that $Y_1^{-/-}$ osteoblasts expressed higher-levels of RANKL ($P<0.01$), leading to increased RANKL/OPG ratio ($P<0.001$).

Paradoxically, functional studies using dentine discs demonstrated that in $Y_1^{-/-}$ cultures there is an inhibition in matrix demineralization. Scanning-electron-microscopy images revealed that $Y_1^{-/-}$ osteoclasts produce poorly demineralized resorption pits. To quantify these differences we have developed a novel computational tool (BonePit program), which allowed us to generate tridimensional (3D) reconstructions of resorption pits and to analyse pits morphological features. 3D analyses showed that $Y_1^{-/-}$ resorption pits displayed a marked reduction in area, volume and depth ($P<0.001$), when compared to WT. In fact, the gene expression of matrix resorption markers, matrix metalloproteinase-9 and cathepsin K, was downregulated in $Y_1^{-/-}$ cultures, suggesting the presence of non-resorbing osteoclasts in $Y_1^{-/-}$ mice.

Together, these data disclosed an important role for Y_1R in osteoclastogenesis, essentially in bone matrix resorption, which may unravel new potential therapies for the treatment of osteoclastic bone diseases.

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The role of LC3 and autophagy in bone resorption by osteoclasts

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The autophagy protein LC3 is necessary for bone resorption by osteoclasts, although it has been suggested that this may be through a novel, autophagy-independent process, by promoting lysosomal fusion at the ruffled border (RB). This process would be analogous to LC3-associated phagocytosis (LAP), in which LC3 is acquired by phagosomes through an autophagy-independent process, and controls phagosome maturation by promoting fusion with lysosomes. We have investigated this possibility by using novel mouse models for monitoring LC3 localisation and a model in which autophagy is selectively ablated. *In vitro*, LC3 localises to the RB in 30% of actively resorbing osteoclasts. Most of these osteoclasts are at an early stage of RB formation; LC3 did not localise to the RB in osteoclasts associated with extensive resorption pits. We further investigated this by using an autophagy-deficient mouse model in which FIP200 is deleted in the myeloid lineage; FIP200 is essential for autophagy, but is not required for LAP. FIP200 null osteoclasts were able to target LC3 to the RB and resorb dentine despite impaired autophagy, indicating that a process similar to LAP, rather than autophagy, controls RB formation. The Rab7 effector Plekhm1 may also be involved in this process; mutations in Plekhm1 cause osteopetrosis, due to the failure of osteoclasts to form RBs and resorb bone. Furthermore, Plekhm1 binds to LC3 and plays a role in autophagosome-lysosome fusion by bridging the membranes of these two vesicle types. Surprisingly, we found little difference in autophagy in osteoclasts derived from patients with Plekhm1 mutations or in mice lacking Plekhm1, compared to family or littermate controls, respectively. By contrast, Plekhm1-deficient osteoclasts exhibited defective RBs and profoundly impaired resorptive activity. These data suggest that Plekhm1 may play a redundant role in autophagy in osteoclasts, but is essential for lysosomal fusion at the RB through interactions with LC3.

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P196

The RECQL4 protein mutated in Rothmund-Thomson syndrome is involved in osteoclast differentiation and function

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Homozygous or compound heterozygous mutations in the *RECQL4* helicase gene are responsible for 65% cases of Rothmund-Thomson syndrome (RTS-type II), a rare premature ageing syndrome. RTS-II patients exhibit poikiloderma and various kinds of bone abnormalities: short stature, congenital radial ray anomalies, bone microarchitecture defects, diffuse or localized osteoporosis and increased risk of osteosarcoma. Mutations in the *RECQL4* gene are also responsible for two other rare diseases, RAPADILINO and Baller-Gerod syndromes. These three syndromes are associated with specific clinical signs but have in common skeletal abnormalities, suggesting that the RECQL4 protein may play a key role in the development and maintenance of bone tissue. RECQL4 belongs to the highly conserved RECQ helicase that play significant roles in DNA metabolic processes and maintenance of genome stability. However, the function of RECQL4 and the cellular pathways in which it is involved remains poorly understood.
The aim of our work is to get a better understanding of the pathophysiology of Rothmund-Thomson syndrome by exploring the role of the RECQL4 helicase in bone biology. Our data suggest that RECQL4 is involved in signaling pathways important for osteoclastogenesis. We found that RECQL4 expression is transiently up-regulated by RANKL during osteoclast differentiation of RAW264.7. We next observed that overexpression of RECQL4 in the RAW264.7 causes osteoclast differentiation even in the absence of RANKL. Osteoclasts overexpressing RECQL4 exhibit increased bone resorption, modified cytoskeleton and accelerated apoptosis compared to control cells. Q-PCR analyses revealed that RECQL4 is able to induce NFATc1, a key transcription factor of osteoclast differentiation. Taken together these data suggest that RECQL4 may be a regulator of osteoclastogenesis. Molecular mechanisms underlying this unexpected function of RECQL4 are under investigation and will be discussed.

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P197

Effects of TGF-β inhibition on osteogenesis and osteoclastogenesis by periodontal ligament fibroblasts from patients with fibrodysplasia ossificans progressiva

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Background
Fibrodysplasia Ossificans Progressiva (FOP) is a progressive disease characterized by periods of heterotopic ossification, often in ligaments. The underlying mechanism is far from clear, partially due to limited access to patient-derived cell models. Periodontal ligament fibroblasts (PLF) from extracted teeth can potentially be used to study deviant bone remodelling processes *in vitro* since these cells are derived from actual ligaments. They further provide a tool to study the hitherto unknown role of ACVR1 mutation, the BMP- receptor that is mutated in FOP patients, in osteoclastogenesis. Several studies suggest a role for TGF-β in FOP osteogenesis. Moreover, TGF-β is a candidate protein for periodontal ligament-induced osteoclast formation.
Objective
To assess the role of FOP PLF in osteogenesis and osteoclastogenesis and the involvement of TGF-β herewith.
Methods
FOP and control PLF ($n=6$ of each) were used in osteogenesis and osteoclastogenesis assays in the absence or presence of TGF-β receptor inhibitor GW788388. Alkaline phosphatase (ALP) activity and alizarin red staining was measured to asses osteoblast differentiation. TRACP staining and multinuclearity in PLF-PBMC co-cultures on cortical bone slices was used as a measure of osteoclast formation. In these cocultures TGF-β expression and RANKL/OPG ratio was measured by QPCR.
Results
Although FOP-PLF displayed a slightly higher ALP activity at 7 days, mineralization was similar at 21 days. GW788388 did not influence mineral deposition in both groups. However, in the osteoclastogenesis cultures, FOP-PLF displayed a two-fold higher osteoclastogenesis on cortical bone slices. Osteoclast formation was inhibited by TGF-β receptor inhibitor GW788388, both in control and FOP cultures. Interestingly, GW788388 inhibited TGF-β expression and had a pronounced effect on the RANKL/OPG ratio in osteoclastogenesis cultures.
Conclusion
Our study emanates an important role for TGF-β in periodontal ligament fibroblasts associated osteoclastogenesis.

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Development of a novel sandwich ELISA to quantify human Tartrate Resistant Acid Phosphatase (TRAP) isoforms 5a and 5b protein in one and the same sample

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Tartrate resistant acid phosphatase (TRAP) consists of two isoforms TRAP 5a and TRAP 5b suggested to exert different functions and clinical relevance. TRAP 5a is a 35 kDa monomer and a potential marker of inflammatory conditions e.g. atherosclerosis and rheumatoid arthritis. TRAP 5b, a heterodimer of 16 and 23 kDa generated by proteolytic cleavage of a repressive loop in TRAP 5a, is used as marker for osteoclast numbers/bone resorption and osteoclast-related pathological conditions. The different functions/pathophysiological relevance of the two isoforms raise the need for a method that separately quantifies protein levels of each isoform. Here we report a sandwich ELISA assay quantifying protein concentrations of TRAP 5a and TRAP 5b in one and the same sample.
The repressive loop present on TRAP 5a but not TRAP 5b was used as immunogen to generate a monoclonal antibody (mAb) 46 specific for TRAP 5a. Full length TRAP 5a was used to raise mAbs recognizing both isoforms. mAb 46 was used as capture antibody in a sandwich ELISA for quantification of TRAP 5a from a TRAP 5a/TRAP 5b protein mixture. After capturing of TRAP 5a, the supernatant free of TRAP 5a was used in another ELISA composed of a capturing mAb recognising both isoforms to quantify TRAP 5b protein. The assay was tested with TRAP 5a/TRAP 5b mixtures and TRAP 5a protein was successfully separated from TRAP 5b protein. In healthy human serum samples the mean concentration of TRAP 5a protein and TRAP 5b protein were determined to be 3.6 ± 0.76 and 0.65 ± 0.31 ng/ml, respectively.
In summary, this novel TRAP 5a/5b sandwich ELISA specifically measures TRAP 5a and 5b protein separately in one and the same sample and thus increases the usefulness of TRAP 5a and 5b protein as markers for different pathological conditions.

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P199

Identification of G protein-coupled receptor 137B (GPR137b) function in mouse and zebrafish osteoclasts

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Analysis of genome wide data such as transcriptomics can identify genes of potential interest to bone biology. These techniques are primarily hypothesis-generating. Determining the role of candidate factors in bone ultimately requires *in vivo* experiments. Gene expression analysis of mouse osteoclast differentiation identified G protein-coupled receptor 137B (*GPR137b*) as highly upregulated. GPR137b is an orphan seven-pass transmembrane receptor of unknown function. We demonstrated co-localization of GPR137b and TRAP in osteoclasts on histologic sections from mouse femurs. To study GPR137b function *in vitro* we used the CRISPR/Cas9 system to generate knock-out murine osteoclast precursor cell lines. *Gpr137b*$^{-/-}$ osteoclasts cultured on mineralized matrix shown increased bone resorbtion activity. To determine whether GPR137b is important for osteoclast function *in vivo*, we used CRISPR/Cas9 to specifically knock-out

the orthologous gene in zebrafish. *Gpr137b* homozygous mutants are viable and fertile and do not display overt morphological defects as adults. However, analysis of pits formation on scales of *gpr137b*$^{-/-}$ *zebrafish* demonstrated an increase in bone resorption, results in line with the data generated with *Gpr137b*$^{-/-}$ mouse cells. These data suggest a role for GPR137b as a negative regulator of osteoclast activity. To determine if the increase in bone resorption in *gpr137*$^{-/-}$ osteoclasts alters skeletogenesis in zebrafish, we are utilizing a robust microCT method we developed to measure and evaluate bone mass and shape. To further study the role of GPR137b in osteoclast function we are assessing osteoclast behavior *in vivo* using a *cathepsinK-dsRed* reporter line in WT and mutant zebrafish.

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P200

RAW264.7 subclones - an *in vitro* model for osteoclast heterogeneity?

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Site-specific osteoclasts (OC) and OC-like cells from different pathologies exhibit heterogeneity e.g. in the resorption machinery, influence of anti-resorptive drugs and response to local or systemic hormones e.g. PTH. The explanation for this is not known but different precursors, local regulation through osteoblasts (OB), osteocytes and extracellular matrix (ECM) could be involved. To study these differences a simple and reproducible cell model is an advantage in pre-clinical studies. For that purpose, RAW264.7, a cell line used for its capacity to form OC-like cells was evaluated for its suitability as a model for OC heterogeneity.
RAW 264.7 subclones were acquired by single cell cloning. Cells were seeded on bone or coated hydroxyapatite wells (Corning Osteo-Assay Surface 96 wells) at 3×10^4 cells/cm^2, then stimulated with RANKL or RANKL + M-CSF at 10 ng/ml for 14 and 7 days respectively, and evaluated by TRAP staining, immunohistochemistry, morphological analysis, gene expression profiling as well as functional assays e.g. capacity to dissolve mineral matrix through acidification.
Gene expression profiling revealed three subclones groups; (i) TRAP^{low}. (ii) TRAP^{high} where TRAP and Cathepsin K were closely correlated. (iii) TRAP^{moderate}/Cathepsin K^{low} but MMP9^{high}. RANKL differentiation of group (i) resulted in a homogenous population of smaller TRAP+ multinucleated cells (MNC) dissolving hydroxyapatite, thus resembling OC. On the other hand, group (ii) resulted in a heterogeneous population of partly larger MNC with lower TRAP expression exhibiting minimal dissolvent of hydroxyapatite, thus resembling multinucleated giant cells. RANKL +M-CSF differentiation of subclones from group (i) and (ii) resulted in inhibition of osteoclast differentiation with no TRAP positive staining, no multinucleated cells and less expression of osteoclast genes markers.
In summary, subclones of the RAW264.7 cell line can be isolated representing macrophage and osteoclast precursors with different phenotypic and functional properties.

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P201

Galectin-1 is involved in osteoclast biology

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Osteolytic bone disease (OBD) is a frequent complication of multiple cancers, such as multiple myeloma. OBD is due to the disruption of balanced bone remodelling, with higher bone resorption due to increased osteoclast activation and osteoblast inhibition. Lectin–glycoprotein interactions have been implicated in osteoclast formation. In the current study, we set out to identify lectins that are involved in osteoclastogenesis and to study their role in this process. We anticipate that this research will lead to the identification of new targets for the treatment of OBD.
Gene Set Enrichment Analysis on publically available microarray data showed a lower expression of galectin-1 (gal-1) in mature osteoclasts compared to monocytic progenitor cells. Gal-1 is a β-galactoside binding protein implicated in myeloma and interestingly already implicated in trophoblast and myoblast fusion. We confirmed a decrease of gal-1 expression during osteoclast formation on the RNA and protein level on primary and cell line-derived osteoclast cultures. Gal-1 localization by confocal microscopy was found to be predominantly membranous in mature osteoclasts while it was ubiquitous in progenitor cells. siRNA-mediated silencing of gal-1 resulted in an increased osteoclastogenesis and larger osteoclasts. Treatment of osteoclast cultures by Anginex, an anti-angiogenic synthetic peptide that targets gal-1, resulted in a reduced osteoclast formation. We observed no difference in osteoclast number in primary cultures derived from gal-1$^{-/-}$ mice compared to wild-type controls. However, gal-1$^{-/-}$ osteoclasts showed a higher resorption activity, corroborated by a higher expression and secretion of tartrate resistant acid phosphatase in these cultures. Taken together, our data implicate gal-1 in osteoclast biology. Analyses of bone parameters by μCT and immunohistomorphometry in gal-1$^{-/-}$ and wildtype mice are currently ongoing. In addition, gain-of-function studies and analysis of signalling pathways in osteoclasts will be performed. Finally, the role of gal-1 in OBD will be studied in the 5TGM.1 murine multiple myeloma model.

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P202

The effect of potassium citrate on human primary osteoclasts *in vitro*

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An increasing amount of scientific evidence suggests that western diet is a risk factor for osteopenia and osteoporosis. Indeed, metabolic acidosis, occurring after high levels of protein intake, may adversely impact on the skeleton by disrupting calcium metabolism, and leads to a decrease of bone mineral density (BMD). Currently, the prevention and treatment of osteopenia and osteoporosis are mainly based on lifestyle modifications (i.e. exercise, quitting smoking and ensuring an adequate daily intake of calcium and vitamin D) and medical therapies (e.g. bisphosphonates, denosumab, and proton pump inhibitors).
Recent studies have demonstrated that potassium citrate supplementation results in BMD increase, sustained improvement of calcium balance, and decrease of bone resorption markers in both postmenopausal women and healthy young adults. Despite promising clinical data on the efficacy of alkaline citrates on calcium metabolism, it is still not clear whether potassium citrate acts only as a buffer or has a direct effect on bone cells.
In this study, we investigated the role of potassium citrate on human primary osteoclasts differentiation and activity in the presence of an acidic microenvironment.
We analyzed the effect of different potassium citrate concentrations on murine osteoclast precursor cells of Raw 264.7 monocyte-macrophage cell line, cultured in pH 6.8 media, in order to mimic a sub-acidosis condition. In particular, we examined Raw 264.7 viability in the presence of increasing concentrations of potassium citrate (0, 5, 7.5, 10, 12.5, 15 and 30 g/l), every 24 h, for a total period of 96 h, using Alamar blue assay. Moreover, we measured pH variation at the same time points.
Our preliminary results demonstrated that only 5 and 7.5 g/l potassium citrate have a alkaline effect at non-cytotoxic doses. These two concentrations will be used to investigate citrate potassium effects on human OC and other bone cells.

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P203

The role of light (TNFSF14) on bone remodeling

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LIGHT (TNFSF14), expressed by different cells of the immune system, binds two trans-membrane receptors: HVEM and LTβR. It is over-expressed in erosive rheumatoid arthritis and lytic myeloma-bone disease and controversial data have been published on its role osteoclast (OC) formation in vitro. Here, we investigated the role of LIGHT on *in vitro* murine osteoclastogenesis model

and bone phenotype in LIGHT$^{-/-}$ mice. Firstly, we showed that murine macrophages stimulated with LIGHT alone did not differentiate into OCs. Consistently, the addition of agonist anti-HVEM and anti-LTβR antibodies did not affect osteoclastogenesis in the same cultures. Interestingly, the presence of LIGHT and sub-optimal RANKL concentration displayed synergic effects on OC formation through the early and sustained activation of Akt, NFκB and JNK pathways.
Secondly, by microCT we found that the femurs of LIGHT$^{-/-}$ mice exhibited a 30% ($P<0.01$) decrease in trabecular BV/TV due to a significant reduction in trabecular thickness and number as well as the increase in trabecular spaces respect to WT mice. Furthermore, a fivefold increase of OC number/bone surface was found in femora from KO mice compared to WT ($P<0.004$).
To investigate the possible molecular mechanism/s responsible for this bone phenotype in LIGHT$^{-/-}$ mice we studied OPG levels in whole bone marrow (BM) extracts from the femurs of these mice and demonstrated a significant reduction in OPG mRNA transcript respect to WT (fourfold, $P<0.001$). Further investigations showed that BM CD8^{+} T cells and B cell subpopulations from KO mice expressed lower levels of OPG compared to those from WT mice. Consistently, LIGHT treatment in a dose dependent manner increase OPG expression in BM CD8^{+} T cells and B-cells.
In conclusion, our results identified LIGHT as a new important modulator of bone remodeling and highlighted a new modulator of OPG expression.

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P204

Fluoride modulates formation and function of bone marrow macrophage-derived osteoclasts in a strain-specific manner

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Osteoclast presents a central role in several inflammatory diseases that are associated to bone destruction. This condition results from increased osteoclastic bone resorption and/or decreased bone formation. Fluoride (F) is widely consumed in the drinking water due to its anticariogenic effect and has been shown to modulate *in vivo* bone metabolism in a strain-specific dependent manner. It enhances bone formation in 129P3/J mice but not in A/J mice. However, its effect on osteoclastogenesis and the strain dependency remain uncertain. Therefore, the objective of this study was to evaluate the effects of F on formation and function of bone marrow macrophage-derived osteoclasts in a strain-specific manner. Thus, bone marrow cells from A/J and 129P3/J were cultivated in α-MEM medium containing M-CSF and RANKL in presence or absence of different concentrations of F for 7 days. The number of osteoclasts was evaluated by tartrate-resistant acid phosphatase (TRAP) staining and their functional responses were evaluated by the tartrate-resistant acid phosphatase (TRAP), metaloproteinase (MMP)-2 and -9 and resorptive activities. In A/J-derived cells, F varying from 10^{-3} to 10^{-7} M significantly increased TRAP activity compared to untreated cells ($P<0.05$). However, no significant alterations were observed in F-treated 129P3/J cells compared to untreated cells. Moreover, control A/J and 129P3/J present similar TRAP activity, suggesting that the strain-specific response was associated to F effect. While the MMP-2 did not change, the activity of osteoclast-produced MMP-9 increased about 30% by 10^{-3} M F in A/J but did not alter in 129P3/J cells. Moreover, 129P3/J-derived osteoclast demonstrated slightly effect on the resorptive function after F treatment whereas osteoclast from A/J mice was significantly altered. Therefore, F enhances osteoclast formation and function in A/J-derived cells but not in 129P3/J- derived cells. The data provide knowledgement of cell-type contribution in the well-known effect of F on the bone metabolism.

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P205

Better understanding the potency and cytotoxicity of different bisphosphonates on murine osteoclast formation and activity: implications for its better clinical use in treatment cancers

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Bisphosphonates are widely used drugs in the fight against osteoclast-mediated bone loss, including osteoporosis and Paget's disease of bone. The first generation of these potent drugs such as clodronate, a non nitrogen-containing bisphosphonate, has been shown to inhibit osteoclast formation and osteoclastic bone resorption both *in vitro* and *in vivo* as well as inducing apoptotic cell death. Recent interest has centred on the effects of more potent nitrogen-containing bisphosphonate zoledronate that appears 10,000-fold more potent. However, their clear dose-dependent anti-resorptive effects on murine osteoclasts as well as their effects on osteoclast formation have not been fully understood. In this study, the effects of different concentrations of zoledronate and clodronate on murine osteoclasts were examined. Mouse marrow cells were cultured with zoledronate and clodronate (1 nM-1 μM) on ivory discs for 8–9 days. Zoledronate was found to significantly reduce osteoclast number and resorption dose-dependently with a complete inhibition at concentrations above 1 μM. Peak effects occurred at 10 μM, where it had strong cytotoxic effect on all marrow-derived cells. Clodronate significantly inhibited osteoclast resorption in all concentration ranges tested, causing two fold and three fold decrease at (1 nM–1 μM) and 10 μM respectively. Clodronate also caused a six fold decrease in osteoclast number at 10 μM. These results suggest that zoledronate is a strong and potent inhibitor of murine osteoclast formation and resorption, as well as highlighting the differences in cytotoxicity and potency between different concentrations of individual bisphosphonates and between bisphosphonate groups. A clear understanding of these effects may have important implications for better clinical use of bisphosphonates in treatment of cancers.

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Cell biology: Osteocytes, mechanobiology

P206

Post traumatic bone defects treated with induced biomembrane technique in a tertiary care trauma centre – technique, results in 6 patients and a review of literature

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Introduction
Segmental bone defects arising from traumatic injuries is a complicated problem with significant long term morbidity. Amputation was the preferred treatment historically. Limb salvage by the Ilizarov technique, vascularized fibular graft, and acute limb shortening was used in the last century with variable results. The problems were long, cumbersome and patient unfriendly treatment regimens, often involving multiple major and minor surgical procedures and numerous complications. More recently Masquelet et al described the use of an antibiotic cement spacer after a thorough debridement for an induced bio-membrane, followed by grafting (with or without mechanical support) within this space with successful outcomes. Similar results could not be reported at multiple centers and it was suggested that the reason was the damage sustained to the biomembrane while removal of the cement. We present our series describing our technique of cementation and cement retrieval which was able to maintain the biomembrane without damage.
Methodology and results
Our study was a retrospective case series (Level of Evidence IV) of six patients with a mean age of 47 with a mean bone defect of 8.8 cm (after debridement). Our technique involved a thorough aggressive debridement of bone and soft tissues, with filling of defect with antibiotic impregnated polymethylmethacrylate bone cement spacer, while maintaining the vascularized soft tissue sleeve, with temporary stabilisation on first presentation. This was followed by second stage removal of cement spacer while maintaining the biomembrane & cancellous bone grafting of the defect with definitive fixation. The average time for bony union was 8 months since first presentation with a mean follow up of 1 year. All patients returned to their preinjury functional level.
Conclusion
The technique of delayed bone grafting with fixation after initial debridement and placement of a cement spacer provides excellent results for patients with large posttraumatic bone segment loss.

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P207

Evidence that osteocyte perilacunar remodelling contributes to polyethylene wear particle induced osteolysis

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Periprosthetic osteolysis (PO) leading to aseptic loosening, is the most common cause of failure of total hip replacement (THR) in the mid- to long-term. Polyethylene (PE) particulates deriving from the wear of prosthesis liners are bioactive and are implicated in the initiation and progression of osteolysis. Evidence exists that cells of the osteoblast/osteocyte lineage respond to PE particles and contribute to the catabolic response by promoting osteoclastic bone resorption. In this study, we hypothesised that osteocytes may also contribute to PO by removing bone from their perilacunar matrix (perilacunar remodelling). Osteocyte responses to ultra-high molecular weight PE (UHMWPE) particles were examined *in vitro* in human primary osteocyte-like cultures, *in vivo* in the mouse calvarial osteolysis model, and in bone biopsies of patients undergoing revision total hip replacement (THR) surgery for PO. Osteocytes exposed to UHMWPE particles showed upregulated expression of catabolic markers, MMP-13, carbonic anhydrase 2, cathepsin K and tartrate resistant acid phosphatase (*TRAP*), with no apparent effect on cell viability, as assessed by Caspase 3 activity. Consistent with this catabolic activity causing perilacunar bone loss, histological analysis of calvarial sections from mice exposed to UHMWPE revealed a significant ($P<0.001$) increase in osteocyte lacunar area (Lac.Ar) compared to sham-operated animals. Furthermore, acetabular biopsies from patients with PO also showed significantly ($P<0.001$) increased osteocyte lacunar size in trabecular bone adjacent to PE particles, compared with osteocyte lacunar size in bone from primary THR patients. Together, these findings suggest a previously unrecognised action of UHMWPE wear particles on osteocytes, which directly results in a loss of osteocyte perilacunar bone. This action may exacerbate the indirect pro-osteoclastic action of UHMWPE-affected osteocytes, previously shown to contribute to aseptic loosening of orthopaedic implants.

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P208

Transgene expression by Dmp1 promoter fragments occurs in various organs

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Analysis of osteocyte function often uses promoter elements of osteocyte-specific genes i.e. SOST or Dentin-matrix-protein 1 (Dmp1) to overexpress genes or the Cre-recombinase for conditional deletion studies. However, evidence suggests that these promoters may not be osteocyte-specific, which would be critical for subsequent data interpretation. To investigate the selectivity of supposedly osteocyte-specific *in vivo* models, we crossed the 8kb-Dmp1-Cre mice (i) with Ai9 tomato reporter mice (Dmp1-Cre^{+};Ai9$^{T/wt}$), and (ii) with mice, in which the expression of the diphtheria toxin receptor (DTR) is controlled in a Cre-inducible manner (Dmp1-Cre^{+};iDTR$^{T/wt}$) to ablate Dmp1-positive cells. Furthermore, we ablated Dmp1-positive cells in mice harboring human DTR (hDTR) regulated by a 10kb-Dmp1 promoter fragment (Dmp1-hDTR). Immunohistochemical staining of tibiae harvested from 8-week old Dmp1-Cre^{+};Ai9$^{T/wt}$ mice revealed a strong tomato expression in all osteocytes and osteoblasts covering endocortical and trabecular surfaces. Furthermore, we detected tomato expression in muscle, brain, testis, and in vessels in the heart, spleen, lung, and intestine. Consistently, histological analyses of bones five days after diphtheria toxin (DT) administration in Dmp1-Cre^{+};iDTR$^{T/wt}$ and Dmp1-hDTR mice revealed an efficient ablation of not only osteocytes but also osteoblasts and lining cells. In addition, DT injection resulted in liver vacuolation, acute kidney necrosis, splenic atrophy, and disturbance of bone marrow composition in Dmp1-hDTR mice, which is consistent with expression of hDTR mRNA in several tissues including muscle, liver, kidney and spleen. Taken together, our results indicate that in 8kb-Dmp1-Cre mice as well as in Dmp1-hDTR mice, expression of the respective transgenes during mouse development and growth is not restricted to osteocytes, but also takes place in other osteolineage cells and in several organs. Our findings therefore suggest that despite the great usefulness of these *in vivo* systems, the expression pattern of the gene of interest should be determined carefully and the results need to be interpreted accordingly.

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Chondrocytes and cartilage

P209

Crosstalk between FLS and chondrocytes is regulated by HIF-2α-mediated cytokines in arthritis

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Rheumatoid arthritis (RA) and osteoarthritis (OA), two common types of arthritis, affect the joints mainly by targeting the synovium and cartilage. Increasing evidence indicates that a significant network connects synovitis and cartilage destruction during the progression of arthritis. We recently demonstrated that hypoxia-inducible factor (HIF)-2α causes RA and OA by regulating the expression of catabolic factors in fibroblast-like synoviocytes (FLS) or chondrocytes. To address the reciprocal influences of HIF-2α on FLS and chondrocytes, we applied an *in vitro* co-culture system using a transwell apparatus. When co-cultured with HIF-2α-overexpressing chondrocytes, FLS exhibited increased expression of matrix-metalloproteinases (MMPs) and inflammatory mediators, similar to the effects induced by tumor-necrosis factor (TNF)-α treatment of FLS. Moreover, chondrocytes co-cultured with HIF-2α-overexpressing FLS exhibited upregulation of *Mmp3* and *Mmp13*, which is similar to the effects induced by interleukin (IL)-6 treatment of chondrocytes. We confirmed these differential HIF-2α-induced effects via distinct secretory mediators using *Il6* knockout cells and a TNF-α-blocking antibody. The FLS-co-culture-induced gene expression changes in chondrocytes were significantly abrogated by IL-6 deficiency, whereas TNF-α neutralization blocked the alterations in gene expression associated with co-culture of FLS with chondrocytes. Our results further suggested that the observed changes might reflect the HIF-2α-induced upregulation of specific receptors for TNF-α (in FLS) and IL-6 (in chondrocytes). This study broadens our understanding of the possible regulatory mechanisms underlying the crosstalk between the synovium and cartilage in the presence of HIF-2α, and may suggest potential new anti-arthritis therapies.

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P210

Thyroid hormone locally interacts with the sympathetic nervous system to control bone linar growth

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It is well known that thyroid hormone (TH) is essential for normal bone growth and development. However, the mechanisms by which TH regulates these processes are poorly understood. Recently, the sympathetic nervous system (SNS) was identified as a potent regulator of bone metabolism. In vivo studies by our group have shown that TH interacts with the SNS to regulate bone mass and structure, and that this interaction involves α_2 adrenoceptor (α_2-AR) signaling. We also identified the presence of α_{2A}-, α_{2B}-, and α_{2C}-AR subtypes in the epiphyseal growth plate (EGP) of mice. In addition, we found that mice with isolated gene deletion of α_{2A}-AR and α_{2C}-AR (α_{2A}-AR$^{-/-}$ and α_{2C}-AR$^{-/-}$) show a disorganized EGP, smaller long bones and a delay in endochondral ossification. Moreover, we found that the EGP of α_{2A}-AR$^{-/-}$ and α_{2C}-AR$^{-/-}$ animals respond differently, than those of wild-type animals, to TH excess and deficiency. These in vivo findings strongly suggest that TH also interacts with the SNS to regulate bone growth and development. Through a long bone organ culture system, the present study aims to investigate if TH interacts with the SNS directly in the skeleton, to regulate bone linear growth and if α_{2C}-AR is involved in this process. We evaluated the linear bone growth of the femur and tibia derived from wild-type (WT) and α_{2C}-AR$^{-/-}$ neonate mice for 12 days. We observed that 10^{-8} M triiodothyronine (T3) treatment for the whole culture period of twelve days significantly decreased bone linear growth of both femur and tibia only in WT animals. The linear growth of the bones derived from KO animals was not affect by T3 treatment. These *in vitro* findings suggest that TH locally interacts with the SNS to control bone linear growth and that this interaction involves α_{2C}-AR signaling.

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P211

Benzo(a)pyrene induce the inflammation, nitrosative stress and matrix degradation in articular cartilage

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Abstract unavailable.

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P212

Histological structure of the mandibular condylar cartilage in rats of various age after 60-day intake of epichlorohydrin

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Objectives
This study is aimed at investigating of histological features of mandibular condylar cartilage (MCC) in rats after 2-month inhalation of epichlorohydrin (ECh) vapors and administration of thiotriazoline (Th) and *Echinaceae tinctura* (ET) as medication.
Methods
The experiment involved 420 male rats (young, adult and senile). The animals were split into the groups: 1st group comprised control animals, the 2nd group comprised the animals that received inhalations of ECh in dosage of ten MPC as a single 5-hour exposure per day, 2nd group – inhalations of ECh and intraperitoneal Th in dosage of 117.4 mg per kg, 4th group – inhalations of ECh and intragastric *ET* in dosage of 0.1 mg of active substance per 100 g of body weight.
Results
In the study we found out that long-term inhalation of ECh (60-day daily 5-hour exposure to ten MPC) results in inhibition of morphofunctional activities (MFA) of the MCC. By the 1st day after ECh discontinue, width of subchondral osteogenesis zone (WSOZ), and amount of primary spongiosa (APS) and number of osteoblasts (NO) in it in young animals decreased in comparison with the control group by 11.33, 9.76 and 11.91% respectively; in adult animals the same values decreased by 11.14, 10.80 and 8.90%; in senile animals those values decreased by 9.07, 6.43 and 8.47% respectively. In readaptation period, in young rats inhibition of MFA of the MCC reduced by the 60th day and few significant changes were found after that term, in adult – alterations persisted up to the 30th day and then started reducing slowly, and in old – did not exhibit restoration signs. By the 60th day after ECh discontinue, WSOZ, and APS and NO in it in young rats decreased in comparison with the control – by 4.90, 3.61 and 3.86% respectively; in adult – the same values decreased by 4.64, 3.54 and 4.23%; in senile – those values decreased by 7.33, 5.90 and 7.50% respectively. In 3rd group restoration of MFA of the MCC in young and adult animals was observed from the 1st up to the 60th day, and in old – from the 7th up to the 60th day. In 4th group of MFA of the MCC in young animals was observed in the period from the 1st up to the 60th day, and in adult and old – from the 15th up to the 60th day of observation. Th thus appeared to be more effective than *ET*.
Conclusions
Application of Th or *ET* reduces negative effects of ECh on the MFA of the MCC. We proved Th to be more effective than *ET*.

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P213

The vacuolar H^+ ATPase V_0 subunit D_2 is associated with chondrocyte hypertrophy and supports chondrocyte differentiation

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In a recent unbiased transcriptomic study of genes associated with equine osteochondrosis, we identified several novel cartilage genes. The current study was undertaken to determine whether these genes are regulated during chondrocyte hypertrophy, and to identify novel hypertrophy-associated genes for further study *in vitro*. Gene expression was investigated by quantitative PCR (qPCR) in different zones of growth cartilage micro-dissected from equine foetal metatarsal bones ($n=22$). Nine novel cartilage genes (*ATP6V0D2, DDX5, GNB1, PIP4K2A, RAP1B, RPS7, SRSF3, SUB1 and WSB2)* were found to be more highly expressed in the hypertrophic zone than in reserve or proliferative zones. Regulation of expression of three of these genes by conditions inducing expression of hypertrophy-associated genes (ITS/AA/T3) was investigated by qPCR in the mouse ATDC5 chondrocyte cell line; *Atp6v0d2, Ddx5* and *Tpi1* were all upregulated under these conditions. Atp6v0d2, the gene encoding the vacuolar H^+ ATPase V_0 subunit d_2, was selected for further study. Immunochemical staining for ATP6V0D2 protein in equine foetal growth cartilage was more intense in the hypertrophic than reserve or proliferative zones; in cultures of ATDC5 cells, staining was more intense in ITS/AA/T3-treated than control cells. Knockdown of *Atp6v0d2* gene and protein expression in ATDC5 cells was achieved using *Atp6v0d2*-targetting siRNA, as confirmed by qPCR ($>50\%$ knockdown, $P<0.05$, in both ITS/AA/T3-treated and control cells) and by immunocytochemistry. In control cells but not ITS/AA/T3-treated cells, the siRNA consistently suppressed expression of *Col2a1* (60%, $P<0.05$). Hypertrophy-associated genes were not differentially expressed between siRNA- and sham-transfected cultures. Knockdown of *Atp6v0d2* expression in ATDC5 cells cultured under hypertrophy-inducing conditions caused a decrease (18%, $P<0.0001$) in nuclear area as well as increases in the number of cells (28%, $P<0.05$) and percentage containing mitotic default (59%, $P<0.0001$). These observations suggest that ATP6V0D2 expression (and presumably vacuolar H^+ ATPase activity) supports differentiation and suppresses proliferation of chondrocytes.

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P214

Hif1alpha leads to chondrodysplasia in MMP-deficient mice

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Hypoxia and the hypoxia-inducible factor 1alpha (Hif1alpha) are known to play critical physiological functions in endochondral bone development. However, their role in abnormal cartilage formation (chondrodysplasia) is unknown. Our goal was to test the possibility that altered oxygen homeostasis, which would result in abnormal Hif1alpha expression and activity, could lead to chondrodysplasia. This was done using matrix metalloproteinase (MMP) 9 and 13 deficient mice, which present a chondrodysplasic phenotype characterized by a modest dwarfism, and a mild bowing on the long bones. This mouse phenotype recapitulates that observed in humans lacking MMP9/13 function. The postnatal growth plates of MMP-deficient mice are characterized by a dramatic accumulation of hypertrophic chondrocytes, and delayed ossification. Interestingly, we observed that a significant increase in hypoxia and Hif1alpha protein levels in fetal growth plates precedes the postnatal growth plate defects of MMP-deficient mice. We used a conditional knockout approach (Col2-CreERt2) to remove Hif1alpha specifically from chondrocytes of newborn MMP-deficient animals. Importantly, removing Hif1alpha from newborn cartilage abolished the growth plate defects in these mice. This result demonstrates that Hif1alpha is responsible for the chondrodysplasic phenotype of MMP-deficient mice. All animal protocols were approved by an animal ethics committee. Mechanistically, our data indicate that increased Hif1alpha activity in MMP-deficient mice slows down hypertrophic maturation of chondrocytes, and increases cell survival of hypertrophic chondrocytes. Together, these cellular effects likely explain the accumulation of hypertrophic chondrocytes and delayed ossification observed in MMP-deficient mice. Hence, our results establish for the first time that extracellular matrix homeostasis maintains oxygen homeostasis to ensure proper skeletal development. Our work also demonstrates the importance of oxygen homeostasis in postnatal skeletal growth, and suggests that Hif1alpha constitutes a pertinent therapeutic target to prevent chondrodysplasia in MMP-deficient patients.

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P215

Nutritional aspects of skeletal development

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While the connection between under-nutrition and growth retardation is well documented, the opposite connection between over-nutrition and bone development was barely studied. For instance, obese children grow faster in height than normal-weighed children, and prospective studies demonstrated an over-presentation of obese children amongst fracture cases. Furthermore, little is known about the direct effect and the underlying cellular and molecular mechanisms of the diet or single nutrients on the cells of the developed bone.
We analyzed in depth the effect of childhood obesity on young bone elongation and bone quality. Multiple complementary *in vivo* models were utilized to characterize in details the growth-plate phenotype as well as the bone structure and mechanical properties. The various models we used are: pharmaceutical inhibition of leptin signaling (by leptin antagonists) and various types of obesogenic diets such as high fat diet (HFD). We found that obesity in young age affected both bone elongation and bone quality. Furthermore, the type of the diet, distinctly from its obesogenic effect, modified bone development and quality. For instance, while HFD based on poly unsaturated fatty acids impairs bone morphology; omega-3 fatty acids improves it.
Our studies demonstrated the involvement of metabolic signals such as adiponectin, leptin and IL1b. We discovered a novel mechanism by which osteocalcin shifts chondrocytes toward glycolytic breakdown of glucose and stimulates their calcification, in a HIF-1α-dependent manner. Based on these findings, we suggest that the metabolic status in obesity and the specific component in the diet affect directly the metabolic state of bone cells, leading to accelerated bone elongation and modified processes of bone formation and resorption. This topic is of tremendous importance for both basic and applicative scientists in the fields of pediatrics, nutrition, endocrinology, bone health and development.

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P216

The role of CANT1 in skeletal development with a mouse model of Desbuquois dysplasia type 1

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Desbuquois dysplasia (DBQD) is a rare recessive chondrodysplasia, characterized by growth retardation, multiple dislocations and advanced carpal ossification. Two forms of DBQD have been described on the basis of the presence (type 1) or absence (type 2) of characteristic hand anomalies. DBQD type 1 is caused by mutations in the Calcium-Activated Nucleotidase 1 gene (CANT1), while DBQD type 2 is caused by mutations in the xylosiltransferase 1 gene.
CANT1 is a nucleotidase of the ER/Golgi that hydrolyzes UDP, suggesting its involvement in protein glycosylation; for this reason its role in proteoglycan (PG) metabolism has been hypothesized.
To better characterize CANT1 role in the etiology of DBQD and in PG synthesis, we generated a Cant1 knock out mouse by excision of exon 3 and 4. The KO mouse showed the same growth defects and hand anomalies of patients. To study PG synthesis, rib chondrocytes were metabolically labeled with 35S-sulfate and the amount of newly synthesized PGs was evaluated. KO cells showed reduced PG synthesis compared to wild types both in presence and in absence of β-D-xyloside, an enhancer of glycosaminoglycan (GAG) synthesis. Gel filtration chromatography of GAGs released from newly synthesized PGs after β-elimination demonstrated that the hydrodynamic size of GAG chains was reduced in KO chondrocytes compared to the controls. Ultrastructural analysis of KO and wild type cartilage and cultured chondrocytes by TEM demonstrated the presence of dilated vacuoli containing electrondense proteinaceus material suggesting a role of CANT1 in protein secretion. Pulse-chase labeling of cells with 35S-sulfate demonstrated reduced PG secretion in mutant cells compared to the controls.
In conclusion, we generated and validated a mouse model to study DBQD type 1 and we demonstrated that CANT1 play a role in PG synthesis.

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P217

Effect of combined sex hormone replacement on bone/cartilage turnover in a murine model of osteoarthritis

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Background
Estrogens act on estrogen receptors distributed in articular cartilages, synovial membrane, and ligaments, which are thought to be related with degenerative changes. Meanwhile, progesterone is known to have a weak anabolic action on bone formation. This study evaluates the effects of estrogen and progesterone hormone on bone/cartilage turnover in ovariectomized (OVX) rats.
Methods
Thirty-five 7-month-old female Sprague–Dawley rats were randomly divided into five groups and then ovariectomized bilaterally except the sham control group. The first and the second group acting as controls did not receive hormonal therapy, the third group received estrogen, the fourth group received progesterone, and the fifth group received combination of both hormones 10 weeks after surgery. Evaluations were done using the serum levels of cartilage oligomeric matrix protein (COMP) for cartilage turnover, collagen type I C-telopeptide (CTX-1) and osteocalcin (OC) for bone turnover at 11, 15, 19 weeks after OVX and histology using the Osteoarthritis Research Society International (OARSI) osteoarthritis (OA) cartilage histopathology assessment system.
Results
Significantly less cartilage degradation (decreased levels of COMP) was found in the combined hormone treated group in comparison with OVX group. Similarly, both hormonal treatment resulted in increased bone formation and decreased bone resorption, i.e., a low overall bone turnover status (decrease in the serum OC and CTX-1 levels).
Conclusions
Combined estrogen and progesterone therapy was found to be convincing in terms of reducing the severity of OA in this experimental model.

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P218

Hypoxia inducible factor-1α directly induces the expression of receptor activator of nuclear factor-κB ligand in ATDC5 chondrocyte

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Reduced availability of oxygen, i.e. hypoxia, could occur during disuse, bone development, and fracture. Cartilage tissue is avascular in nature and the microenvironment of cartilage is hypoxic. Hypoxic regulation of gene expression generally involves activation of the hypoxia-inducible factor (HIF) transcription pathway. Receptor activator of nuclear factor-kappaB ligand (RANKL) is an osteoblast/stromal cell derived essential factor for osteoclastogenesis. Hypoxia-induced enhanced osteoclastogenesis via increased RANKL expression has been well demonstrated in *in vitro* system using osteoblasts. However, during bone development, hypoxic signaling regulation mechanism in mediating osteoclastogenesis, that is, osteoclast recruitment mechanisms after cartilage calcification by hypertrophic chondrocytes has remained unclear. In the present study, we investigated whether hypoxia regulates RANKL expression in ATDC5s, murine chondrocytes and HIF-1α mediates hypoxia-induced RANKL expression by transactivating RANKL promoter.
The expression levels of RANKL mRNA and protein, as well as HIF-1α protein, were significantly increased under hypoxic condition in ATDC5s. Constitutively active HIF-1α alone significantly increased the levels of RANKL expression in ATDC5s under normoxic conditions, whereas dominant negative HIF-1α blocked hypoxia-induced RANKL expression. To further explore to find if HIF-1α directly regulates RANKL transcription, a luciferase reporter assay was conducted. Hypoxia significantly increased RANKL promoter activity, whereas mutations of putative HIF-1α-binding elements in RANKL promoter prevented this hypoxia-induced RANKL promoter activity in ATDC5s. Our results suggest that RANKL is a target gene of HIF-1α and that hypoxia plays a role in osteoclastogenesis during bone development, at least in part, through the induction of RANKL expression in chondrocytes.

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P219

Characterization of COMP in zebrafish

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COMP (Cartilage Oligomeric Matrix Protein) is a member of the thrombospondin family of extracellular matrix proteins and also referred to as thrombospondin-5. Mutations in COMP lead to chondrodysplasias. Here we studied COMP in zebrafish (Danio rerio) which is a powerful vertebrate model organism. *In silico* analysis revealed that only one COMP coding gene exists in zebrafish and that the domain structure of COMP is fully conserved. Inspection of gene locus and phylogenetic analysis confirmed that zebrafish COMP is indeed the ortholog of mammalian COMP. Zebrafish COMP was recombinantly expressed, used for immunization of rabbits and guinea pigs and the antisera were affinity purified against recombinant COMP. Immunohistochemistry using affinity purified antibodies showed COMP expression already in early stages of development. Starting from 10 hpf COMP is detected in the somites. This was unexpected, as such an early expression is not known for mammalian COMP. However, *in situ* hybridization confirmed the unexpected early expression. As in mammals at later stages COMP is found in cartilage, tendons and skin. Nevertheless, in comparison with the strongly expressed cartilage-specific protein matn3a, COMP is only moderately expressed in cartilage. Western blot analysis carried out from protein extracts revealed that zebrafish COMP also forms disulfide-liked pentamers. By Crispr/Cas9, we are aiming at producing mutant zebrafish lines that can be used as models to gain further insight into the pathomechanism and treatment of chondrodysplasias.

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P220

Role of matrilin-3 in the development of osteoarthritis

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Introduction
The matrilin-3 polymorphism T298M has been described to be associated with hand osteoarthritis in the carpometacarpal joint 1 (CMC1) and with spinal disc degeneration. *In vitro* studies revealed that expression levels, processing and secretion of matrilin-3 T298M were similar to the wt protein. Structural analysis indicated an impact on the formation of collagen II/IX/XI heterofibrils, whereas the binding to collagen was not affected. We have now generated a knock in mouse (matrilin-3 T298M/T298M) to investigate the skeletal development and its applicability as a monogenetic model for osteoarthritis.
Methods
The secretion of matrilin-3 in primary chondrocytes was investigated via immunoblot. Micro-CT was used to analyse bone architecture of the forepaw at the age of 4, 8 and 18 weeks. Sequential extraction of newborn femoral head cartilage was performed to assess matrix integrity. Immunostaining was used to investigate the matrix composition in forepaw (newborn, 4, 8, 18 weeks) and spine (newborn, 4 weeks).
Results
Secretion of mutant matrilin-3 by primary chondrocytes was comparable to wt protein and also in tissue there were no signs of intracellular retention. Micro-CT measurements showed no differences in the forepaw. The extractability of the mutant matrilin-3 seems to be increased. The staining of the spine showed an increased collagen IX signal. Analysis of the forepaw by immunohistochemical staining revealed the first evidence of osteoarthritis in the CMC1 joint of matrilin-3T298M/T298M mice at the age of 18 weeks.
Discussion
Previous data suggested that the polymorphism has an impact on fibrillogenesis which might lead to alterations in stability and stiffness of the ECM. The reduced anchorage of matrilin-3 indicated by an increased extractability of the mutant protein might contribute to altered biomechanical properties of the tissue. These alterations in the ECM could be responsible for an early onset of osteoarthritis in transgenetic matrilin three animals.

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P221a

miR-214: a novel regulator of chondrogenesis?

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Skeletogenesis is an intricate process controlled by numerous transcriptional factors, hormones and signalling pathways. Recently, microRNAs emerged as important players in skeletogenesis but, only few were identified and most of their targets remain unknown. Previous works showed that miR-199a-2/214 cluster is essential for skeletal development and that miR-214 inhibits bone formation in mammals. However, data regarding its skeletal role in other vertebrates is scarce and its role in chondrogenesis is still unknown. Here, we aimed to uncover the potential role of miR-214 in chondrogenesis, by analysing its transcriptional regulators and mechanisms of action, using an *in vivo* accepted model for vertebrate skeletogenesis, the zebrafish, and an *in vitro* chondrocyte-like cell model, the ATDC5 cell line.
First, we characterized miR-214 expression throughout zebrafish development, by *in situ* hybridization, and identified an association with skeletal formation, since miR-214 is particularly expressed in zebrafish mineralizing cartilaginous structures. Moreover, reporter gene assays and chromatin immunoprecipitation studies led us to conclude that both human and zebrafish miR-199a-2/214 promoters are active and similarly regulated in chondrocyte cells and that Ets1 regulates miR-214 transcription in ATDC5 cells. Importantly, overexpression of miR-214 in ATDC5 cells mitigated chondrocyte differentiation probably by targeting Atf4. Two key skeletal markers, Mgp and Osteocalcin, were simultaneously decreased upon miR-214 overexpression in ATDC5 cells, suggesting that mineralization, the late stage of chondrocyte differentiation, is compromised. Interestingly, like Osteocalcin, we show that Mgp transcription could be controlled by the complex Atf4-Runx2-Satb2. Our data indicates that miR-214 exerts a key role in skeletal development, not only by inhibiting osteogenesis but also by affecting chondrogenesis. In this process, we hypothesize that miR-214 affects the expression of proteins relevant for bone and cartilage formation (Mgp and Osteocalcin) by targeting a transcriptional activator of both molecules, Atf4.

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P221b

Vitamin D supplementation for 12 months in older people prevents bone loss and suppresses parathyroid hormone levels

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Background, subjects and methods
Vitamin D insufficiency in older people in the UK is common and may cause secondary hyperparathyroidism and bone loss. In a randomised, double blind intervention trial to optimise "Vitamin D status in Older People' (VDOP) three oral dosages of vitamin D_3 (12 kIU, 24 kIU or 48 kIU/month) were given for 12 months to 375 participants aged over 70 years (ANOVA) adjustment for covariables with results below presented in ascending dose order.
Results
Baseline characteristics, including BMD, 25OHD and PTH did not differ between the groups (all $P > 0.05$) with a mean (s.d.) 25OHD of 35.5 (20.0) nmol/l, rising by 14.3 (12.6), 25.3 (18.0) and 40.6 (20.2) nmol/l respectively (ANOVA, $P < 0.001$) and resulting in plasma 25OHD levels ≥ 25 nmol/l in 99%, 100% and 100% and ≥ 50 nmol/l in 63%, 83% and 100% respectively.
BMD at total femur and neck of femur did not change over 12 months (ANOVA for ΔBMD and ANCOVA comparisons between doses, all $P > 0.05$). However, PTH decreased for all three doses by -2.9 (18.4), -2.9 (18.1) and -10.6 (15.4) pg/ml respectively (ANOVA $P < 0.001$ and when adjusted for age, sex, weight, height and baseline 25OHD ANCOVA, $P < 0.001$). There were no cases of hypercalcaemia, renal stones or adverse events attributable to the intervention and there were no group difference in the number of falls.

Summary
We conclude that monthly supplementation with 12 kIU, 24 kIU or 48 kIU/month vitamin D is safe and associated with improvements in vitamin D status in this population at risk of vitamin D deficiency. No significant decrease in BMD was seen with any dose, despite an anticipated decrease of ~0.6% in this age/geographical group.
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Energy metabolism and bone, fat and bone

P222

Changes in circulating osteocalcin concentration in overweight and obese adult women having normal glucose and hemoglobin A1c levels

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Osteocalcin (OC) plays crucial roles in glucose homeostasis. OC level in the circulation appears to be associated with glucose and fat metabolism – Although still it remains controversial if it are the levels of total OC and/or under-carboxilated OC (ucOC) which present such association. The present study evaluated the levels of ucOC, total OC, leptin and insulin, in 226 overweight or obese (degree I and II) adult non-diabetic women, having levels of glucose (80–110 mg/dl) and hemoglobin A1c (HbA1c $<5.7\%$) within normal range. Women, aged 20–86 years were divided in four groups according to their menopausal status: premenopause (PreM) (<45 ys); peri-menopause (periM) (45 and <50), early menopause (EM) (50–65 ys) and late menopause (LM) (>65 ys). Body weight and height were used to calculate BMI score for determining overweight and the degree of obesity. Calcium intake (CaI) was estimated by a semi-quantitative food frequency questionnaire. Serum levels of glucose, cholesterol, triglycerides, HbA1c were carried out automatically using standard laboratory methods. Enzyme immunoassays were used for serum ucOC (ng/ml), OC (ng/ml), leptin (ng/ml), insulin (uUI/l) levels determination. Levels of 25hydroxyvitamin D (25OHD) (ng/ml) were assayed by a competitive protein-binding method. Some results (mean ± S.D.) are shown in the table.

Menopausal status	Ucoc	OC	Insulin	Leptin
Overweight				
preM	1.96±1.08	29.9±2.7	9.2±3.9	11.1±3.3
periM	2.75±1.22	31.3±7.3	5.8±1.1	9.7±1.7
EM	2.20±1.34	35.1±5.3	6.1±1.7	9.7±1.1
LM	3.81±1.89	29.1±4.6	5.2±1.4	7.3±2.5
Degree I obesity				
preM	2.21±0.98	30.0±2.21	11.7±1.9	17.8±4.3
periM	2.93±0.78	31.6±5.9	11.8±2.4	18.2±2.4
EM	3.41±096	37.1±9.5	9.8±1.5	20.9±5.2
LM	3.81±0.96	24.9±4.5	11.8±3.2	17.7±6.3
Degree II obesity				
preM	4.08±0.41	19.9±8.6	16.0±6.4	43.7±8.8
periM	4.50±0.22	21.6±11.3	12.5±2.5	25.3±6.14
EM	4.11±0.6	21.7±4.7	10.3±1.7	27.1±4.4
LM	5.52±1.7	26.9±5.2	12.8±1.8	31.0±8.2

Irrespectively of the mean age, the levels of ucOC, insulin and leptin increased as the BMI increased while OC levels decrease in degree II obese women.
For a same BMI, ucOC showed a tendency to increase while OC did not change with increasing age.
Conclusions
Although further studies are needed, the present study showed that the levels of ucOC and not the levels of total OC are BMI-dependent in normoglucemic non-diabetic women. Grants: PROINCE E-006 (UNLaM 2015–2016) and CONICET.
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P223

Cyclic AMP/protein kinase A signalling downregulates Dlx5 expression via inducing C/Ebpβ in 3T3-L1 preadipocytes

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Distal-less homeobox 5 (Dlx5) is a transcription factor that enhances osteogenic differentiation of mesenchymal stem cells via upregulating the expression of Runx2 and other osteoblast phenotypic genes. We have previously demonstrated that Dlx5 also downregulates adipogenic differentiation of mesenchymal stem cells and that insulin decreases expression levels of Dlx5 via increasing expression levels of miR-124, a microRNA targeting 3′UTR of Dlx5. However, the mechanism of Dlx5 down-regulation by adipogenic stimuli other than insulin has not been clarified yet. In this study, we examined the effect of cAMP/protein kinase A signalling on the expression of Dlx5 in 3T3-L1 preadipocytic cells. Activation of cAMP/protein kinase A signalling by 3-isobutyl-1-methylxanthine (IBMX), 8CPT-cAMP, foskolin or prostacyclin significantly downregulated the expression levels of Dlx5 mRNA and protein. These stimuli induced CREB phosphorylation and increased expression levels of CREB and C/EBPβ. Forced expression of CREB or C/EBPβ suppressed Dlx5 expression, whereas knock-down of C/EBPβ prevented IBMX from downregulating Dlx5 expression. Overexpression of C/EBPβ significantly decreased the luciferase activity of promoter reporter containing 3 kb murine Dlx5 promoter sequence. Chromatin immunoprecipitation assays demonstrated that IBMX treatment or CREB overexpression increased the binding of C/EBPβ but decreased the binding of RNA polymerase II to the Dlx5 promoter region. These results suggest that cAMP/protein kinase A signalling attenuates Dlx5 transcription via enhancing the binding of C/EBPβ to the Dlx5 promoter.
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P224

Osteocalcin downregulates pancreatic lipase expression in GPRC6A dependent manner

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We previously elucidated that β-adrenergic blockade attenuates high fat diet induced obesity development and such an effect is associated with suppressed pancreatic lipase (PNLIP) expressions. In the present study, we tested whether β-adrenergic blockade downregulates bioactive osteocalcin secretion in osteoblasts, thereby reduces PNLIP secretion in pancreatic acinar cells.
Forty of male 6wk old C57BL/6 mice were assigned into control diet (CON) and a high calorie diet (HIGH) group. In each diet group, mice were treated with vehicle (VEH) or with propranolol, a β-adrenergic antagonist (BB; 0.5 g/l in drinking water) over 12 weeks.
Expression levels of ESP, a tyrosine phosphatase which downregulates the carboxylation of osteocalcin, in HIGHVEH mice femur were higher vs in CONVEH mice, however, this increment was attenuated by β-blockade in HIGHBB animals. Reductions in serum undercarboxylated bioactive osteocalcin (ucOC) level in HIGHVEH mice was mitigated in HIGHBB mice. In MC3T3E1 osteoblasts, upregulated ESP expression, followed by downregulated osteocalcin expression in isoproterenol, β-adrenergic agonist, treated cells, was attenuated by propranolol treatment. *In vitro* experiment using primary pancreatic acinar cells and 266-6 cells, pancreatic acinar cell line, PNLIP expressions decreased when the cells were treated with ucOC. ucOC also attenuated isoproterenol induced mouse PNLIP promoter activity. G protein-coupled receptor (GPRC6A), a candidate receptor for mediating the response to ucOC in the bone-pancreas endocrine loop, was highly expressed in mouse pancreatic tissues and in 266-6 cells. Knockdown of GPRC6A by siRNA suppressed downregulating effect of unOC on PNLIP expressions, proposing that GPRC6A mediates responses to osteocalcin in pancreatic acinar cells. In summary, β-adrenergic blockade rescued bioactive osteocalcin secretion in osteoblasts, thereby osteocalcin suppressed PNLIP expressions in pancreatic acinar cells. OCN downregulates PNLIP expressions in GPRC6A mediated manner. These data sheds a light on the crucial endocrine role of the skeleton regulating body energy metabolism.
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P225

Association between bone turnover markers and leptin in girls with adolescent idiopathic scoliosis (AIS)

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The link between scoliotic deformity and bone metabolism in adolescent idiopathic scoliosis (AIS) has not been well researched. Moreover, the data concerning the cross-talk between leptin level and bone markers in this group of patients are lacking. The objective of this study was to correlate the extent of scoliotic-curve severity with the bone turnover and leptin level in girls with AIS. The study encompassed 77 AIS girls, aged 14.7±2.17 years. Scoliotic curve severity assessed by Cobb's angle was categorized as mild (10–19°) moderate (20–39°) or severe (≧40°). Corrected height, weight, waist and hip circumferences were measured and body mass index (BMI), corrected height Z-score, BMI Z-score and waist/height ratio (WHtR) and were calculated for the entire group. Body composition parameters: fat mass (FAT), fat-free mass (FFM) and predicted muscle mass (PMM) were determined using a bioelectrical impedance analyzer. Bone turnover markers (osteocalcin (OC) and amino terminal of collagen cross-links NTx) and leptin levels were assessed in serum. Multiple regression analysis showed that, OC, NTx (negatively with $P<0.05$) and leptin (positively with $P<0.01$) were significantly associated with curve severity in AIS girls. Moreover, Cobb's angle was positively correlated with W/HtR ($P<0.01$)and FAT ($P<0.05$). One-way analysis of variance (ANOVA) revealed significant differences in leptin ($P<0.05$ *vs* mild only), OC ($P<0.05$ *vs* mild and moderate) and W/HtR ($P<0.01$ and $P<0.05$ *vs* mild and moderate respectively) between the three scoliotic severity subgroups. OC was significantly lower in the severe AIS subgroup, while leptin and W/HtR were significantly higher. Significant correlations between leptin and anthropometrical parameters as BMI z-score and W/HtR were shown. Leptin level correlated also significantly with BMI z score ($P<0.001$), W/HtR ($P<0.0001$) and body composition parameters ($P<0.000001$). Moreover, there was a significant negative correlation between NTx and leptin level ($P<0.05$). Bone metabolism in AIS girls seems to altered and significantly related to the scoliotic curve severity. Leptin may be a crucial link in the cross-talk between bone turnover and body composition in this group of patients. Further studies concerning this topic are needed.

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P226

Osteocalcin interacts with brain-derived neurotrophic factor, nerve growth factor but not oxytocin in the regulation of bone, energy, brain and reproductive functions

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Osteocalcin, the neurotrophins BDNF/NGF and Oxytocin(Oxt) have pleiotropic effects on energy metabolism, bone mass, reproduction and brain functions suggesting a coordinated regulation. The carboxylated osteocalcin(Ost) acts on bone, while the uncarboxylated Ost shows hormone-like actions. NGF regulates female fertility elevating LH, $Ost^{-/-}$ mice show high LH in spite of decreased testosterone. BDNF/NGF-Oxt-Ost interactions was investigated by RT-PCR measuring mRNA levels of NGF, BDNF, Oxt, Ost and their receptors p75NTR/NTRK1, TRKb, Oxtr and Gprc6a in brain, bone, WAT/BAT and reproductive organs, of 3 months old female and male mice using brain and bone as positive controls, respectively. NGF and p75NTR expression is 50% higher in BAT than brain and are down-regulated in WAT and bone in both genders. Ost and Gprc6a are upregulated in bone and brain, down-regulated 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 ovaries/uterus, but down-regulated in testes. p75NTR is respectively 300, 100 and 50% higher in testis, ovaries and uterus than brain. NTRK1 is down-regulated in all tissues. The Gprc6a is expressed in testes, not in ovaries and uterus. BDNF and TRKb are down-regulated in reproductive organs. Oxt is expressed in brain and with minor extend in bone in either genders while Oxtr in ovaries, a significant expression level is observed in fat and bone. The up-regulation of NGF and related-receptors in fat is consistent with NGF as energy regulator. The up-regulation of p75NTR matches the Gprc6a in testes, while inverse correlation of NGF and BDNF in fat and bone, shows these exerting opposite effects on leptin with BDNF regulating bone. BDNF-NGF-Ost genes interaction is observed. BDNF may regulate the exclusive actions of carboxylated Ost on bone, while NGF modulates the uncarboxylated Ost hormonal actions.

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P227

The role of interleukin-6 and tumor necrosis factor alpha gene in fat and bone communication

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Objective
To comparing the role of interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) in fat and bone communication.
Methods
Male wild type (WT) mice, IL-6 knockout ($IL\text{-}6^{-/-}$) mice, and tumor necrosis factor alpha (TNF-α) were fed with either standard diet (SD) or high fat diet (HFD) for 12 weeks. Bone mass and bone microstructure were evaluated by micro-CT. Gene expression related to lipid and bone metabolisms was assayed with real-time qRT-PCR. All animal experiments were performed in accordance with the guidelines for the care and use of laboratory animals and were approved by the Institutional Animal Care and Use Committee at the West China Hospital, Sichuan University.
Results
On the SD, the levels of plasma total cholesterol (TC) and low density lipoprotein (LDL-C) in $IL\text{-}6^{-/-}$ mice and $TNF\text{-}\alpha^{-/-}$ mice were higher than that in WT mice, and $IL\text{-}6^{-/-}$ mice showed higher level of TC while $TNF\text{-}\alpha^{-/-}$ mice showed higher level of LDL-C. HFD increased the levels of TC and LDL-C in all three strain mice, and the change was more obvious in $TNF\text{-}\alpha^{-/-}$ mice. The changes of adipocyteogenesis were also different, HFD increased the expression of *PPAR-r* and *leptin* mRNA levels in only WT mice, but not in $IL\text{-}6^{-/-}$ mice and $TNF\text{-}\alpha^{-/-}$ mice.
On the SD, $IL\text{-}6^{-/-}$ mice exhibited significantly higher trabecular thickness (Tb.Th), but $TNF\text{-}\alpha^{-/-}$ mice exhibited significantly higher trabecular number (Tb.N) than WT mice. After HFD feeding, trabecular bone volume fraction (Tb.BV/TV), Tb.N and Tb.Th were down-regulated, and trabecular space (Tb.Sp) was up-regulated in WT mice. Changes of these parameters were in similar tendency in $IL\text{-}6^{-/-}$ mice, but they were in opposite tendency in $TNF\text{-}\alpha^{-/-}$ mice.
Conclusion
Both of IL-6 and TNF-α played a critical role in the HFD-related trabecular bone loss; and their roles in regulate fat and bone metabolism significant differently.

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P228

Effects of roux-en-Y gastric bypass surgery on bone quality: a pilot study

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Roux-en-Y gastric bypass surgery (RYGBP) is one of the leading surgical treatments for morbid obesity and leads to significant long-term weight loss, diabetes remission, decreased cardiovascular events, and reduced mortality. RYGBP is, however, also implicated in increased fracture risk, mainly due to higher bone turnover rates and malabsorption. In the present study we used Raman microspectroscopic analysis to determine bone quality (an important

determinant of bone strength) in paired iliac crest biopsies from eight morbidly obese patients before (three of which suffered from type 2 diabetes), and nine morbidly obese patients 1 year after RYGBP (five of which were type 2 diabetics in remission); seven of these cases were paired biopsies. The bone quality indices assessed included mineral/matrix ratio, nanoporosity (a surrogate for tissue water content), as well as glycosaminoglycan (GAG), lipid, and pyridinoline (PYD) content. Results obtained before and after RYGBP were compared using paired t-tests. In the osteoid, GAGs were significantly lower after RYGBP (−10%, $P<0.05$), consistent with the reported higher bone turnover rates in these patients following surgery. Within mineralized bone matrix, RYGBP resulted in a higher GAG content (possibly indicative of an altered canalicular network; +20%, $P<0.05$), and significantly lower nanoporosity (−57%, $P<0.01$) and PYD values (+2%, $P<0.05$), also consistent with higher bone turnover rates following surgery. The mineral/matrix ratio remained unchanged. Regression analysis of all individual data before and after RYGBP revealed significant alterations in the relationship between GAG and lipid content, potentially due to diabetes remission following RYGBP. In summary the results of the present pilot study indicate that RYGBP results in significant changes in bone quality irrespective whether the patients suffered from diabetes as well, which are consistent with the reported higher bone turnover rates prevalent following surgery.

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P229

Impaired adipose tissue inflammation results in increased bone mineral density in mice

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Obesity, diabetes, and high fracture risk is linked, but the mechanism is unclear. However, impaired acute adipose tissue inflammation may be a common denominator for these conditions. Impaired adipose tissue inflammation leads to low adipogenesis, insufficient adipose expansion, and signs of diabetes.

The aim of this study was to investigate if the low adipogenesis induced by impaired adipose tissue inflammation is coupled to increased osteoblastogenesis, and if this results in high BMD and fracture risk, as seen in patients with type 2 diabetes.

High fat diet (HFD) was fed for 11 weeks to female and male RID transgenic (tg) mice, which have an impaired adipose tissue inflammation, and wildtypes. Body composition, including BMD, was determined throughout the study by dual energy X-ray absorptiometry. At termination, femur was collected for analysis by peripheral quantitative computed tomography. This study was approved by the ethical committee for animal experiments in Gothenburg.

Male RID tg mice had higher total BMD at 4 (+5.0%, $P=0.002$), 8 (+5.4%, $P<0.001$), and 11 (+3.6%, $P=0.006$) weeks of HFD, as well as higher lumbar BMD at 4 (+10.6%, $P=0.002$), 8 (+16.8%, $P<0.001$), and 11 (+7.5%, $P=0.02$) weeks of HFD, compared with wildtypes. Male RID tg mice also had higher femoral trabecular BMD (+9.1%, $P=0.02$) and cortical content (+9.7%, $P=0.02$) than wildtypes. For female mice, the bone phenotype was not apparent until 11 weeks of HFD, when female RID tg mice had higher lumbar (+16.5%, $P<0.001$), but not total, BMD than wildtypes. Female RID tg mice also had higher femoral trabecular BMD (+10.8%, $P=0.01$) and cortical content (+8.2%, $P=0.005$) than wildtypes.

In conclusion, male and female mice with an impaired adipose tissue inflammation had higher BMD than wildtypes. However, the effect was delayed in females. The fracture risk and osteoblastogenesis remains to be investigated.

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P230

The interrelationship of 25-hydroxyvitamin D and osteocalcin on glycolipid metabolism: a cross-sectional study in type 2 diabetics

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Context

As bone metabolic markers, recent studies indicated vitamin D and osteocalcin (OC) were also involved in energy metabolism. While the exact relationship between vitamin D and OC in regulating energy metabolism is unclear. One of the possibilities is that: low vitamin D could lead to secondary hyperparathyroidism and following high bone turnover, which would enhance the quantity of bioactive OC.

Objective

Our aim was to study whether OC mediated the metabolic effect of vitamin D, along with their interaction on glycolipid metabolism in a cross-sectional study.

Method

A total of 701 Chinese type 2 diabetic patients, aged from 27 to 93, were recruited. Spearman correlation and linear regression analysis were performed. OC and OC * 25(OH)D were added to the regressions in order to study the interaction effect.

Results

The inverse associations of 25(OH)D with HbA1c(beta = −0.013, $P=0.001$), FPG (beta = −0.019, $P=0.001$), 2hPG (beta = −0.020, $P=0.018$), TG (beta = −0.005, $P=0.021$) and positive associations with 2hCP (beta = −0.014, $P=0.004$), HDL (beta = 0.002, $P<0.001$) and HOMA2-%B (beta = 0.250, $P=0.003$) were independent of PTH and OC in type 2 diabetics. The significant interaction of 25(OH)D and OC on HOMA2-%B (P for interaction = 0.0362) were observed in this study.

Conclusion

No evidence suggests that OC mediates the vitamin D metabolic effect in our study. 25(OH)D combining with OC may have greater facilitation to beta-cell function.

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P231

Osteocalcin transgenic mice reveal aspects of the bone/glucose axis, as well as powerful suppression of ectopic osteocalcin protein production

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Rationale

Mice deficient in osteocalcin show an impaired glucose tolerance, however, the exact mechanism involved have yet to be fully elucidated. We aimed to overexpress osteocalcin to determine its effect on glucose homeostasis and explore the signalling pathway involved. We wished to expand upon previous preliminary data suggesting that this pathway involve neuropeptide Y (NPY) signalling.

Objective

To analyse the *in vivo* and *in vitro* phenotype of three transgenic models over expressing osteocalcin: i) OcnCre/Ocntg- in osteocalcin-producing cells (osteoblasts/ osteocytes), ii) NPYCre/Ocntg- in NPY expressing cells (hypothalamus, SNS, osteoblasts) and iii) PanCre/Ocntg- in all cells.

Methods and results

Glucose tolerance tests demonstrated that increased osteocalcin expression improves glucose tolerance. At 10 weeks, OcnCre/Ocntg displayed a significant increase in glucose tolerance; however this difference was lost as mice age. In OcnCre/Ocntg serum osteocalcin levels were slightly elevated at 16 weeks (mean (S.D.), ng/ul control 35.2(1), NPYCre/Ocntg 38.7(2), $P<0.05$), however, glucose tolerance was not different at this age, or at 22 weeks. In NPYCre/Ocntg, the more 'neural' model, improved glucose tolerance was maintained in 15-week-old mice, without change in serum osteocalcin (mean (S.D.), ng/ul control 39.0(2), NPYCre/Ocntg 35.1(2), ns). Interestingly, all transgenic models displayed powerful suppression of osteocalcin protein production, despite increased osteocalcin expression by the transgene. This was confirmed in PanCre/Ocntg, which did not display an increase in serum osteocalcin at 15 weeks, (mean (S.D.), ng/ul control 38.1(1), PanCre/Ocntg 35.1(2), ns), despite a marked increase in expression in non-skeletal tissues, and no increase in osteoblastic osteocalcin protein despite greater expression in primary osteoblastic cultures. This suppression of protein production attenuated any long-term changes in glucose tolerance.

Conclusions

These data indicate that osteocalcin released from bone cells is involved in modulating glucose homeostasis. Osteocalcin within NPY expressing cells increases the glucose modulatory effect, without a circulatory circuit, suggesting the potential for direct signalling of osteocalcin in neural cells. Osteocalcin protein production is powerfully limited in an anatomical, temporal and differentiation-specific manner.

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P232

Significant correlation between vasculopathy and bone mineral density in T2DM: new light shed on diabetic osteoporosis

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Purpose
Osteoporosis (OP) aggravates with vasculature degeneration and improvement of vasculature could prevent OP. We, thus, hypothesize that vascular structure and function play an important role in the development of OP. Type 2 diabetes mellitus (T2DM) patients are vulnerable to vasculopathy, so T2DM were involved in this project to study the correlation between vasculopathy and bone mineral density (BMD).
Methods
A total of 427 (180 in OP group; 247 in non-OP group) postmenopausal women with T2DM were consecutively involved. Data on baseline characteristics were collected. Vasculopathy severity and BMD were respectively evaluated by carotid ultrasonography (GE L05, LOGIQ 5) and dual energy X-ray absorptiometry (Hologic QDR4500W). Patients were assigned into two categories (OP = 1 or non-OP = 2) according to BMD. A semiquantitative scale score (score 0–4, the score higher, the plaques more sever) using University of Washington criteria was used to assess the severity of carotid plaques. Estimated glomerular filtration rate (eGFR) was calculated by the CKD-EPI equation. Logistic regression analysis was used to study the association between BMD and vasculopathy. All subjects agreed to participate in the study with written informed consent.
Results
Compared with non-OP group, OP group was illustrated with high scores of carotid plaque (1.67 ± 1.55 vs 1.27 ± 0.42, $P = 0.008$) and decreased eGFR (79.32 ± 23.40 vs 84.03 ± 25.19 ml/min/1.73 m^2, $P = 0.002$). Moreover, notably higher levels of osteocalcin and C-terminal telopeptide fragments of type I collagen (CTX) were observed in OP group compared with non-OP group. Multivariate logistic regression analysis in all patients revealed that BMD was negatively correlated with age ($r = -0.104$, $P < 0.001$), and carotid plaque score ($r = -0.132$, $P = 0.023$), while positively correlated with BMI ($r = 0.175$, $P < 0.001$), HDL ($r = 1.254$, $P = 0.009$) and eGFR ($r = 0.19$, $P = 0.038$).
Conclusion
The results demonstrate a positive correlation between BMD and vasculopathy in postmenopausal women with T2DM, providing evidence for further investigation of T2DM-relative OP.

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Genetics and Epigenetics

P233

Differentially methylated regions in gene enhancers of mesenchymal stem cells from osteoporotic patients

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Osteoporosis (OP) is characterised by reduced bone mass, due to an insufficient osteoblast-mediated bone formation, unable to replace the bone tissue removed by osteoclasts. Mesenchymal stem cells (MSCs) are multipotent cells capable of differentiating into osteoblasts, adipocytes and chondrocytes. Epigenetic marks like DNA methylation could influence the differentiation potential of these cells into osteoblasts and, consequently, the risk of OP. To explore this hypothesis, we analysed the methylome and the transcriptome of MSCs derived from patients with osteoporotic hip fractures (OP) and control individuals with osteoarthritis (OA).
MSCs were obtained from the femoral bone marrow ($n = 20$) and their nature confirmed by the expression of surface markers and the ability to differentiate into osteoblasts and adipocytes. DNA methylation was analysed with the HumanMethylation450K Bead Chip (Illumina®); RNA transcripts were analysed by NGS.
MSCs from patients with osteoporotic fractures showed 4417 hypermethylated and 4621 hypomethylated CpG sites, in comparison with OA. Of these, there were 40 differentially methylated regions (DMRs) located at CpG islands, 217 located at promoters, 129 located at gene bodies and 1684 located at gene enhancer regions. Enhancer DMRs were associated with genes that were over-represented in several bone-related pathways, like the Wnt pathway or osteoblast differentiation and ossification pathways.
The RNAseq analysis showed 174 genes significantly over-expressed and 355 under-expressed in fractures in comparison with OA. Many differentially expressed genes were also associated with DMRs at their enhancers. In fact, 30 genes overexpressed in fractures showed enhancer's DMRs (14 hypermethylated, 16 hypomethylated). On the other hand, 67 under-expressed genes had DMRs (48 hypermethylated and 19 hypomethylated).
In conclusion, we found a number of DMRs (mainly in enhancer regions) in MSCs of patients with OP and OA that are likely to influence their capability to differentiate into bone forming cells. Validation experiments are underway.

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SNP regulation of miRNA expression and its association with osteoporosis

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Biogenesis and function of microRNAs can be influenced by genetic variants in the pri-miRNA sequences leading to phenotype variability. The aim of this study was to identify osteoporosis-related SNPs by affecting the expression levels of mature microRNAs.
The first approach was to perform an association analysis of putative functional SNPs located in pri-miRNA sequences of bone-related microRNAs with the lumbar spine and femoral neck (FN) bone mineral density (BMD). In this regard, OSTEOMED2 cohort was created by recruiting postmenopausal women from several Spanish regions ($n = 2183$).
Multivariate linear regression models were fitted to assess the association between genotyped SNPs and BMD. Potential confounders considered for adjustment were densitometer devices, body mass index and age.
Two SNPs, rs6430498 in the miR-3679 and rs12512664 in the miR-4274, were significantly associated with FN BMD. Allele A (minority allele) for the rs6430498 and allele A (majority allele) for the rs12512664 were found associated with lower BMD values.
Further, we measured these BMD-associated microRNAs in whole trabecular bone from osteoporotic FN fractures comparing to non-osteoporotic bone by qPCR. Both microRNAs were found overexpressed in fractured bone.
Finally, a correlation was observed among genotypes of rs6430498 and rs12512664 and the expression levels of the miR-3679 and miR-4274 in human osteoblastic cells, respectively. In both cases, the allele A was associated with higher microRNA expression levels.
In conclusion, two novel osteoblast-expressed microRNAs, miR-3679 and miR-4274, have been associated with BMD and its overexpression could contribute to the osteoporotic phenotype. These findings open new areas for the study of regulation abnormalities in bone disorders as well as for identifying possible new treatment targets.

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P235

Periostin serum levels and gene polymorphism are associated with bone microarchitecture

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Background
We previously reported that serum periostin levels are determined by additive genetic effects. Whether serum levels and/or SNPs in the periostin gene (Postn) contribute to bone microstructure however remains unknown.
Aim
To investigate the association between periostin levels, Postn SNPs, bone-mass and bone microarchitecture in a cohort of postmenopausal women.
Methods
A total of 648 postmenopausal women from the Geneva Retirees Cohort were analyzed for six periostin SNPS (rs9547952, rs9603226, rs7322993, rs9576308, rs7338244, rs9547970). Periostin serum levels were determined by ELISA. Areal bone mineral density (aBMD) was measured by dual-energy X-ray absorptiometry at radial, lumbar and femoral sites (Discovery A, Hologic® inc, Waltham, MA, USA). Distal radius, tibia volumetric BMD and bone microstructure were measured by high-resolution peripheral quantitative computed tomography (XtremCT, Scanco Co, Bruttisellen, CH). Regression analyses were carried out to determine the association between periostin serum level and SNPs with bone traits.
Results
Mean age of the cohort was 64 ± 1.4 years. Periostin levels were positively associated with 1/3 radius aBMD (beta $= 0.10$, $P = 0.009$) and negatively with radius cortical porosity (beta $= -0.09$, $P = 0.02$). Periostin SNPs rs7322993, rs9576308, rs7338244, rs9547970 were associated with aBMD at lumbar, femoral and ultradistal radius sites. Those SNPs were also associated with radius and tibia microarchitecture (trabecular number and cortical porosity). Once adjusted for age, height, weight and years since menopause only rs7322993, rs9576308, rs9547970 remained significantly associated with both lumbar aBMD ($P = 0.02 - 0.04$) and radius trabecular number ($P = 0.008 - 0.04$). There was no association between periostin serum level and periostin SNPs.
Conclusion
Periostin levels and SNPs are significantly associated with aBMD and with radius bone microstructure. If confirmed in independent cohort, these results would contribute to understand the genetic determinants of bone fragility.

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P236

Interaction between periostin gene (Postn) and other gene polymorphism involved in periostin expression and activity on bone microstructure in humans

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Background
Periostin is a matricellular protein involved in bone modeling and remodeling through the modulation of WNT-β catenin signaling in osteoblasts and osteocytes.
Aim
To investigate the interaction between polymorphisms of six periostin SNPs and other gene polymorphism involved in periostin expression and activity on periostin serum levels and bone microarchitecture in a cohort of postmenopausal women.
Methods
A total of 648 postmenopausal women from the Geneva Retirees Cohort (GERICO) were analyzed for six periostin SNPS (rs9547952, rs9603226, rs7322993, rs9576308, rs7338244, rs9547970) and for several SNPs in BMP2, CTNNB1, ESR 1, ESR 2, LRP5, LRP6, PTH, PTH R, SPTBN1, SOST, TGF B, TNFRSF11A and WNT 16. Periostin serum levels were determined by ELISA. Distal radius, tibia volumetric BMD and bone microstructure were measured by high-resolution peripheral quantitative computed tomography (XtremCT, Scanco Co, Bruttisellen, CH). Two ways ANOVA was carried out to assess the SNPs effects and their interaction on cortical porosity or periostin serum levels.
Results
ESR1 SNP rs851982, LRP5 SNP rs648438 and TNFRSF11A SNP rs2957137 were associated with periostin serum levels (P values range 0.03–0.0004) as well as with cortical porosity (P values range 0.04–0.005). Periostin SNP rs9547970 was also associated with cortical porosity at radius and tibia ($P = 0.04$).
Furthermore, we identified an interaction between LRP5 SNP 648438 and periostin SNP rs9547970 on radial cortical porosity ($P = 0.005$), and on periostin serum level ($P = 0.01$). In particular, lower periostin serum levels and higher cortical porosity were associated with periostin SNP 954790 GG and LRP5 SNP rs648438 CC and CT.
Conclusion
Gene polymorphism in the estrogen receptor, WNT and RANK pathway are associated with serum periostin levels and cortical microstructure. LRP5 interacts also with Postn polymorphism on bone microstructure. If confirmed in independent cohort, these data confirm the complexity of genetic determinant of bone fragility that involves periostin.

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P237

Association among oxidative stress, Wnt signaling and trabecular bone microstructure in osteoporosis and osteoarthritis

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Experimental studies suggested that both, oxidative stress and the Wnt pathway, are important factors in the regulation of bone remodeling. Thus, low antioxidant levels and elevated markers of Wnt pathway inhibitors (sclerostin) levels are associated with a reduced bone mineral density and increased risk of osteoporotic fracture. Whether oxidative stress and the Wnt pathway are related to fracture risk is poorly understood.
M&M: Cross-sectional study in 21 subjects divided into three groups: seven osteoporotic hip fracture (age: 75 ± 5) (OP); eight osteoarthritis, undergoing hip replacement, (71 ± 4) (OA) and six OA $\leq$ 55 years old.
We carried out hip BMD (DXA-Hologic Discovery) and microstructural and biomechanical characteristics of trabecular bone (Micro-CT-Scan Sky 1172). In macerated trabecular bone, we quantified gene expression of catalase, GADD45 (oxidative stress genes), connexin 43, cyclin D1 (Wnt pathway genes), Runx2, osteoprotegerin (OPG) and sclerostin (SOST) by qPCR.
The results are analyzed statistically with the Kruskal–Wallis and Dunn's *post hoc* and correlations by Pearson coefficient (SPSS 22.0), $P \leq 0.05$.
Results
Osteoporotic subjects have an increased expression of catalase and GADD45 in trabecular bone, suggesting increased oxidative stress in these patients regardless of age and sex.
We also observed a significant increase in the expression of genes involved in the Wnt pathway, connexin 43 and cyclin D1, Runx2 and OPG, in OP group. We found no differences in the SOST gene expression.
As expected, BMD values are statistically lower in the OP subjects and they have a worse biomechanic and microstructure bone.
Conclusion
These results suggest that the trabecular bone from patients with osteoporosis have a higher activity of oxidative stress and alterations in the Wnt pathway and osteogenic genes expression.

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P238

Genetic variants at the Wnt/β-catenin and oestrogen receptor signalling pathways are associated with low bone mineral density in dancers

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Purpose
Research suggests that dancers are at higher risk of developing low bone mineral density (BMD) compared with the general population. However, the associated factors contributing to low BMD in dancers are not fully understood. We aimed to assess the association of single-nucleotide polymorphisms (SNPs) in the Wnt/β-catenin and oestrogen receptor (ER) signalling pathways with low BMD in dancers.
Methods
A genetic association study was conducted in 151 female and male dancers and 151 controls matched for age and sex (18.2 ± 10.8 years vs 18.2 ± 10.7 years). Participants were stratified into different groups according to bone mass outcomes: low BMD (*Z*-score < -1.0 for adults and *Z*-score < -2.0 for adolescents) and normal BMD (*Z*-score ≥ -1.0). Eleven SNPs of the Wnt/β-catenin (*SOST*: rs851054, rs851056, rs10534024, rs4792909, rs9902563; *LRP5*: rs3736228, rs2306862, rs682429, rs491347, rs3781590, rs2508836, rs643892, rs312786) and ER (*ESR1*: rs2234693, rs9340799; *ESR2*: rs1256030, rs960070) pathways were genotyped and evaluated for association with low BMD at the forearm, lumbar spine (LS) and femoral neck (FN). A false discovery rate correction was used to claim significance ($P < 0.02$).
Results
Comparing controls with normal BMD and dancers with low BMD, *ESR1* rs9340799 A allele significantly increased the odds of low BMD in dancers by 1.95-fold (95% CI = 1.09–3.51, $P = 0.0204$) at the forearm, 2.32-fold (95% CI = 1.24–4.32, $P = 0.0059$) at the LS, and 2.45-fold (95% CI = 1.26–4.74, $P = 0.0052$) at the FN. *LRP5* rs2508836 C allele was also associated with an increased risk of low BMD in dancers at the LS (OR = 6.90, 95% CI = 1.27–37.49, $P = 0.009$). Haplotype analysis revealed that the blocks GCGT and GCAG at the *LRP5* gene significantly increased the odds for low BMD in dancers at the LS and forearm (OR = 8.97, 95% CI = 1.14–70.31, $P = 0.0368$ and OR = 6.43, 95% CI = 1.33–31.14, $P = 0.0207$).
Conclusion
Genetic variants at the Wnt/β-catenin and ER pathways are associated with low BMD in dancers.

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P239

Search for BMD-related variants of DKK1 and SOST by resequencing in the BARCOS cohort

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In a meta-analysis by Estrada *et al*. (2012), 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 two inhibitors: *DKK1 and SOST*.
To better understand the role of these genes in BMD determination and fracture susceptibility, we aimed to explore their allelic architecture by resequencing all coding exons and flanking regions 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 ones were genotyped in the complete BARCOS cohort and, where appropriate, tested for association.
Resequencing of *DKK1* and *SOST* identified 11 and 3 SNVs, respectively. Half of them had frequencies above 1%, and the rest were observed in only one or two samples, each. Only the rare variant c.*752C > T, in *DKK1*, was novel. One low-frequency variant in *DKK1* showed significant differences between the genotype frequencies of the two extreme groups (rs74711339, $P = 0.0224$).
This SNP and two SNPs (*DKK1*: rs1569198, SOST: rs17882143) and one rare variant (*SOST*: rs570754792) with a potential biological function were genotyped in $n = 1625$ women from the BARCOS cohort. We tested the association of the three SNPs with LS-BMD and nominal significant results were only obtained for rs17882143. This SNP is a missense variant (p.V10I) and in our cohort it is in strong linkage disequilibrium with rs4792909 (the 'GWAS hit'). Regarding the rare variant rs570754792, it was found in heterozygosity in only three women, whose values were below the mean BMD of the BARCOS cohort. It lies in a putative transcriptional regulation site.
In conclusion, our results suggest that the *SOST* p.V10I missense variant may play role in BMD determination. Functional studies to test this hypothesis are underway.

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P240

Analysis of the polyalanine repeat polymorphism in the *RUNX2* gene in relation to bone mineral density and fracture risk in Maltese postmenopausal women

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Introduction
Runt-related transcription factor 2 (RUNX2) is a major transcription factor essential for the regulation of osteoblast and chondrocyte differentiation, hence affecting skeletogenesis, bone and cartilage formation. The RUNX2 protein has unique consecutive polyglutamine and polyalanine repeats (Q/A) which are important for its transactivation function. Several variants within the *RUNX2* gene have been implicated in osteoporosis and fracture susceptibility.
Aim
To evaluate the association of an 18 bp deletion within the polyalanine tract (17A > 11A; rs11498192) with bone mineral density (BMD) at lumbar spine (LS) and hip, and with different types of low-trauma fractures.
Methods
A case-control collection of 1043 Maltese postmenopausal women was used. Women who suffered a fracture were classified as cases whereas those without a fracture history were included as controls. Genotyping was performed by polymerase chain reaction and odds ratios (OR) were computed using logistic regression analysis adjusted for confounders.
Results
RUNX2 alleles were observed at a frequency of 0.90 and 0.10 for the 17A and 11A alleles, respectively, and which were found to be in Hardy-Weinberg equilibrium. Carriers of the 11A allele were found to have a twofold increased risk of osteoporosis at the total hip (adjusted OR: 2.1 [1.1–3.9], $P = 0.02$) and to a lower extent at the femoral neck (adjusted OR: 1.7 [1.1–2.5], $P = 0.02$). No association was observed for the LS BMD. Heterozygosity for the 11A allele was also associated with an increased hip fracture risk which was not attenuated after adjusting for BMD (adjusted OR: 2.2 [1.1–4.8], $P = 0.03$).
Conclusion
Results from this independent replication study indicated that the *RUNX2* 11A variant predisposes to reduced BMD and increased fracture risk in a site-selective manner in Maltese postmenopausal women. The deletion is thought to alter the secondary structure of RUNX2 thereby affecting its transcriptional ability.

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P241

Identification of a novel locus on 2q13 of large effect size which predisposes to clinical vertebral fractures independently of BMD: the GEFOS consortium

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Vertebral fractures are the most common complication of osteoporosis, but little is known about the genetic determinants of susceptibility. Here we present the results of a genome wide association study in 1553 postmenopausal women with clinical vertebral fractures and 4340 controls, with replication in 667 cases and 2105 controls. A locus tagged by a less frequent variant (rs10190845, A-allele MAF = 0.05) was identified on chromosome 2q13 as a strong predictor of clinical vertebral fracture ($P = 1.27 \times 10^{-8}$) with a large effect size (odds ratio 1.75, 95% CI 1.4–2.1). Three other loci were identified on chromosomes 1p31, 11q12 and 15q11, associated at suggestive level ($P < 5 \times 10^{-6}$). All were novel loci that had not previously been associated with bone mineral density (BMD) or clinical fractures. Analysis of 71 variants that had been associated with spine BMD or fractures at a genome wide significant level in other studies identified eight that were significantly associated with vertebral fractures in the present study after Bonferroni correction ($P < 7 \times 10^{-4}$). Bioinformatic analysis of the 2q13 locus identified several potentially functional SNPs which were associated with expression of the positional candidate genes *TTL* and *SLC20A1* in whole bone tissue, none of which is known to play a role in bone biology. Our study illustrates that some predisposing variants for clinical vertebral fractures overlap with known genetic determinants of susceptibility to osteoporosis whereas others are unique. The study has cast new light on the genetic architecture of clinical

vertebral fractures and has identified a variant with one of the largest effect sizes so far described in osteoporosis genetics.

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P242

A family with Paget disease of bone caused by a novel mutation of *hnRNPA2B1* gene

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Paget disease of bone (PDB) is a common metabolic bone disease characterized by increased bone resorption and disorganized bone formation which can affect single or multiple sites of bone. Although the exact cause of PDB is still controversial, genetic factor is considered to play an important role in PDB. The causative gene of classical PDB was identified as *Q8STM1* gene. Familial expansile osteolysis caused by the mutation of *TNFRSF11A*(*RANKL*) gene and juvenile PDB caused by mutation of *TNFRSF11B*(*OPG*) gene have similar pagetic pathology of bone. Multisystem proteinopathy (MSP), a newly proposed syndrome including inclusion-body myopathy (IBM), PDB, frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS), is mainly caused by mutation in *VCP* gene. In 2013, a new casual gene for MSP was identified as *hnRNPA2B1* gene. This may give part explain to the inherited PDB which is negative for mutation in *TNFRSF11A/B* and *Q8STM1* gene. We investigate a Chinese family with multiple affected individuals with Paget disease of bone but no member shows symptoms of IBM, FTD or ALS. Three patients were evaluated clinically, biochemically and radiographically. To screen for the responsible mutation, whole-exom sequencing was conducted in the proband, another patient and a normal individual from the family and revealed a novel heterozygous missense mutation of *hnRNPA2B1* gene (c.929C>T, p.P310L) in the two patients and then verified in all the affected individuals. We describe a novel missense mutation in *hnRNPA2B1* gene in a big pedigree affected with Paget disease of bone whose members do not present other manifestation of multisystem proteinopathy, such as IBM, FTD and ALS.

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P243

Geometric morphometrics: a mathematical tool to compare morphological traits based on *SOST* dysfunction in mice

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Introduction
Genetic disorders are usually characterised by subjective morphological traits. Mouse models should represent the human situation but it is difficult to translate morphology traits from one species to another. Thus, there is a demand to objectively assess morphological traits. Geometric morphometrics is a mathematical tool to evaluate morphology based on normalised three-dimensional coordinates. Here we use geometric morphometrics to compare the morphology of *SOST* knockout (*SOST* KO) mice with respective wild type (WT) mice and relate the symptoms to human sclerosteosis.
Materials and Methods
We analysed the morphological differences between six mice skulls lacking sclerostin and six respective wild type controls (ethically approved). A total of 27 landmark coordinates per skull were obtained based on microcomputed tomography surfaces. Generalized Procrustes analysis superimposes the skull shapes, allowing computing and visualizing morphological dissimilarities and size. Statistical evaluation of morphological differences was done by principal component analysis. We further computed the asymmetry by reflected relabelling and calculated the mandibular prognathism by comparison of distances.
Results
Generalized Procrustes analysis revealed that *SOST* KO mice have a larger centroid size (WT: mean 1587 ± 11; SOST KO: 1654 ± 15), and are more asymmetric (*SOST* KO: 0.000669; WT: 0.000429 [Procrustes sum of squares]) than WT mice. Principal component analysis showed that SOST KO caused curved skulls and a smaller diameter of the foramen magnum in relation to WT mice. Moreover, *SOST* KO mice have dental and skeletal mandibular prognathism in relation to their WT littermates.
Conclusion
Geometric morphometrics is a mathematical tool to discover morphological dissimilarities in the skull of genetically manipulated mice. This method allows visualizing morphological differences in mice that represent human genetic disorders.

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P244

NBAS is the gene mutated in two patients affected by Acrofrontofacionasal Dysostosis type 1

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Acrofrontofacionasal Dysostosis type 1 (AFFND1) is a rare human syndrome (estimated prevalence lower than 1:1,000,000), characterized by bone abnormalities in addition to other multiple congenital anomalies and intellectual disability. Only four AFFND1 families, three of Brazilian and one of Indian origin, have been described so far and an autosomal recessive pattern of inheritance has been suggested. The patients are severely affected: the main clinical features are intellectual disability, short stature, facial and skeletal abnormalities characterized by cleft lip/palate, campto-brachy-poly-syndactyly and marked anomalies of foot structures.
Exome sequencing in the Indian family, comprising two affected siblings and their parents identified a putative candidate variant in an intronic region of the *Neuroblastoma Amplified Sequence* (*NBAS*) gene. This variant was confirmed by Sanger sequencing in the patients and their parents. Since it was located close to an acceptor splice site and predicted to impact on the splicing process, its effect was verified on the cDNA of the patients, and indeed this genomic change was found to cause skipping of exon 48 and premature termination of transcription. Evaluation of expression of the *NBAS* gene in the mouse osteoblast lineage by semiquantitative RT-PCR showed that NBAS expression was almost completely absent in undifferentiated murine Mesenchymal Stem Cells (mMSCs), but greatly increased in mature osteoblasts derived from them. In addition, immunohistochemical analysis of NBAS during murine fetal life starting at embryonic day (E) 11.5 (that is, when skeletal elements begin to form) showed a pattern of expression in agreement with the multiple abnormalities presented by AFFND1 patients in keeping with its possible role in developmental processes.
Further investigations are ongoing in order to unravel the role of the *NBAS* gene in the pathogenesis of AFFND1 and in general in developmental pathophysiology.

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P245

Circulating microRNAs in postmenopausal women with osteoporosis and vertebral fractures

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Circulating microRNAs (miRNAs) are currently being investigated as novel biomarkers for osteoporosis and osteoporotic fractures. The aim of the present study was to investigate the differential expression of specific circulating micro

RNAs known to regulate bone metabolism and homeostasis in postmenopausal osteoporotic women with and without vertebral fractures. For the analysis, miRNAs were isolated from the serum of 24 osteoporotic patients with at least one moderate vertebral fracture and 24 osteoporotic women without vertebral fractures. Twenty postmenopausal women with normal BMD and with no previous history of any kind of fracture were also included in the analysis as controls. From the 14 miRNAs that were selected we identified seven miRs, namely miR-21, miR-23a, miR-124, miR-2861, miR-29a, miR-29b, miR-29c that were significantly deregulated in the serum of osteoporotic patients compared to controls. Two of them (miR-124 and miR-2861) were significantly upregulated while miR-21, miR23a, miR29a, miR29b and miR-29c demonstrated a significantly lower expression in the serum of osteoporotic patients compared to controls. In the sub-group analysis in the osteoporotic group of patients, miR-21, miR-29a, miR-29b and miR-29c were significantly lower in osteoporotic fractured patients compared to osteoporotic patients without fractures. MiR-218, was upregulated in the fractured osteoporotic patients compare to non-fractured, but without reaching statistical significance. This study shows that the expression pattern of specific miRNAs in the serum of osteoporotic patients at increased risk for vertebral fractures may be used as a diagnostic tool for further optimizing fracture risk assessment.

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P246

In a population based association study: IAPP gene variants are not associated with bone phenotypes in elderly women

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Skeletal strength, maintained through bone remodelling, is regulated through complex communication networks between bone cells and other tissues including endocrine cells. Bone also functions as an endocrine organ in its own right. The peptide hormone amylin (or Islet Amyloid Polypeptide (IAPP)), has links to both bone and energy metabolism. A member of the calcitonin family of peptides, it is co-secreted with insulin from pancreatic β-cells and is linked with diabetes-associated complications. As many diabetic patients are osteopenic, and amylin-deficient mice have low bone mineral density (BMD), variation in the IAPP gene may be hypothesised to play a role in the development of osteoporosis.

The objective of the study was to determine the association between single nucleotide polymorphisms (SNPs) in the IAPP gene on DXA measured BMD, quantitative ultrasound (QUS) of the calcaneous and incident fracture in the population based OPRA cohort of 75 year old women. Written informed consent was obtained from all participants. Four SNPs in IAPP, identified from HAPMAP were successfully genotyped by Taqman in 964 women. Statistical association with skeletal phenotypes was analysed (SPSS v22).

There was no association between IAPP SNPs and BMD at any skeletal site or QUS variables. Compared to women with the rs5484 SNP variant "TT" genotype, the risk of incident osteoporotic fracture was elevated in both "CT" and "CC" genotypes. However the association was not statistically significant (CT: OR 1.8 (CI: 0.98–3.5); $P=0.058$) (CC: HR 1.7 (CI: 0.94–3.2); $P=0.080$).

In conclusion, while the data does not support an association between the IAPP variants analysed and BMD or risk of fracture in this cohort, replication studies in larger sample sizes may be warranted to fully explore the contribution of genetic variation in IAPP to bone phenotypes at the population level.

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P247

Common variants in Rspo 1,2 and 3 do not associate with BMD in stratified subpopulations of the Odense Androgen Study and mutations in these genes are not a common cause of craniotubular hyperostosis

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The R-spondins are a family of four small, secreted agonists of the Wnt signaling pathway. Growing evidence from both *in vitro* studies and *in vivo* models supports the major role of these proteins in the skeletal development processes. In humans, common genetic variation in the RSPO3 gene has been associated with BMD in large scale GWAS study.

This study aimed at further investigation of the genetic and functional contributions of the R-spondins in bone biology. We performed an expression study in differentiating osteoblastic KS483 cells revealing that only RSPO1-3 are robustly expressed during this process. After prioritizing RSPO1-3 for genetic testing, we conducted a candidate gene mutation screening in a population of patients suffering from different forms of craniotubular hyperostosis in search for rare, activating mutations in the genes but no potentially pathogenic variants were detected. To further study the relation between common variants in R-spondin genes and BMD we decided to perform a candidate gene association study in two subpopulations of Odense Androgen Study (OAS) stratified based on BMD. This study was performed to confirm the association signals detected in GWAS and search for causal variants with increased resolution. Unfortunately, no significant associations were detected in our cohort. Interestingly, one of the polymorphisms, namely rs140821794 (p.Met16Val) was only detected in a single individual out of the low BMD OAS subpopulation. We performed a dual luciferase reporter assay to test the functional relevance of this variant but could not confirm the causal role of this polymorphism.

Taken together, our data suggest that despite robust expression during bone formation and clear Wnt signaling activation in the presence of the R-spondins, the genetic variation in these genes does not contribute greatly to the genetic determination of bone mass. These findings do not diminish the potential utility of the R-spondins as targets for modulating the Wnt signaling pathway in future treatments of osteoporosis.

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P248

Identification and functional validation of microRNA expression in human bone tissue

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miRNAs are epigenetic regulators of gene expression, increasingly recognised as prominent regulators of bone metabolism. Following our *in vitro* studies of miRNAs profiles in osteoblasts we extended the research to human bone samples. Our aim was to find and functionally validate miRNAs differentially expressed in osteoporotic and osteoarthrotic bone with potential for targeting genes relevant in bone metabolism.

Trabecular bone tissue samples were collected from 44 osteoporotic patients (OP) with hip fracture and 44 patients with primary osteoarthrosis (OA) of the hip during arthroplastic surgery following ethical approval. Fifteen autopsy cases served as a control group (C). In the discovery stage Nanostring nCounter technology was used to obtain the expression profile of 800 miRNAs from 5 OP and 5 OA bone samples. qPCR was used for the validation of differentially expressed miRNAs and their predicted targets.

Nine miRNAs were differentially expressed in OP and OA groups in the discovery stage. miR-195-5p and miR-204-5p were selected for validation in the whole group of 103 patients. Additionally, their potential target genes relevant in bone metabolism were identified using three target prediction tools and were measured. There was a significant difference in miR-195-5p but not miR-204-5p levels among the tested groups ($P<0.0001$). Furthermore, there was a negative correlation between the levels of miR-195-5p and possible target genes *ELOVL6* ($P=0.001$), *GSTCD* ($P<0.0001$) and *MYB* ($P=0.035$). Their gene expressions were also significantly different in the three groups with the highest levels in OP followed by OA and C groups ($P<0.0001$ for all three genes).

Our results are the first to identify miR-195-5p as a potential biomarker in OP and OA, which has only recently been demonstrated to inhibit osteoblast differentiation. Moreover, negative correlations with *ELOVL6*, *GSTCD* and *MYB* gene expression represent evidence of interaction. Further functional confirmations of predicted target genes are underway.

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P249

Identification of epigenomic regulators of osteoblast function

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Molecularly characterized epigenetic networks that control bone formation and are altered during aging are necessary to uncover new potential targets for osteoanabolic therapy. Our contribution to the iBONE consortium is to identify osteoanabolic epigenomic regulators by screening which are involved in osteoblast phenotype and differentiation. This study will be done by a 3 step approach including: siRNA screening for epigenomic regulators of osteoblastic differentiation and validation, identification of target genes by ChIP and functional analyses.
We are currently establishing the siRNA screening. To do this, we chose a colorimetric alkaline phosphatase assay and the determination of Runx2 transcriptional activity or activity of the canonical Wnt/β-catenin pathway using specific reporter plasmids to quantitate osteoanabolic activity. Reporter constructs contain the firefly luciferase cDNA under the control of either Runx2 (8OSE2Tk-luc) or the Wnt/β-catenin signaling pathway responsive elements (TOPflash).
The hFOB1.19 and the FhSO6 cell lines have been stably transfected with the reporter constructs using TALE nuclease or CRISPR/Cas9 technology. The FhSO6 cell line is more differentiated than hFOB, having higher alkaline phosphatase activity and reduced type 1 collagen expression. TALEN and CRISPR/Cas9 have been used to insert the two reporter constructs and their respective negative controls (Tk-luc and FOPflash) into the AAVS1 docking site by homologous recombination in both cell lines. In parallel we have developed an alkaline phosphatase assay for high-throughput screening in 96 well-plates.
We will use a custom siRNA library targeting 347 epigenetic regulators which contain siRNAs against histone and chromatin modifiers, DNA methylation partners and histone chaperones.
Currently, we have FhSO6 and hFOB stably transfected clones and their characterization is ongoing. Some results on the siRNA screening will be presented at the meeting. Through these efforts, we expect to identify some novel epigenomic regulators of osteoblast function.

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P250

Advancing maternal age at childbirth is associated with less favourable trabecular bone mineral density and tibial cortical bone geometry in young adult male offspring

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Background
Advancing maternal age at childbirth has been associated with a higher risk of pregnancy complications and with adverse short-term and long-term offspring health outcomes, but little is known about the effects of increasing maternal age on offspring bone acquisition.
Objective
To investigate associations of maternal age at childbirth with offspring bone parameters in healthy men at the age of peak bone mass.
Methods
We used cross-sectional data from 689 subjects participating in a population-based sibling pair study including healthy men aged 25–45 years. Data include maternal age at childbirth, offspring birth weight, adult weight and height, and offspring DXA and pQCT-derived areal and volumetric bone parameters and cortical bone geometry. Cross-sectional associations were investigated using linear mixed-effects modeling with adjustment for offspring age, height and weight.
Results
Maternal age at childbirth was 27.0±4.7 years and correlated inversely with areal bone mineral density (aBMD) and bone mineral content (BMC) at the lumbar spine ($\beta=-0.09$, $P=0.011$ and $\beta=-0.01$, $P=0.017$), as well as with trabecular volumetric BMD (vBMD) at the distal radius ($\beta=-0.10$, $P=0.014$). No associations of maternal age were found with aBMD or BMC at the total hip or femoral neck, nor with cortical vBMD at the proximal radius or tibia. However, increasing maternal age was associated with smaller cortical thickness ($\beta=-0.13$, $P=0.002$), cortical area ($\beta=-0.08$, $P=0.024$), and cortical over total bone area ratio ($\beta=-0.11$, $P=0.010$) as well as with larger endosteal circumference ($\beta=0.10$, $P=0.010$) at the tibia. No associations of maternal age with cortical bone geometry were observed at the radius.
Conclusions
Advancing maternal age at childbirth might adversely affect the acquisition of peak bone mass and geometry in male offspring, with in particular less favourable trabecular bone mineral density and tibial cortical bone geometry. Whether this results from altered placental function or from epigenetic changes due to lifestyle factors remains to be established.

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P251

Establishment of an *in vivo* model to examine the osteoanabolic epigenome

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Increasing bone formation is an effective approach to prevent osteoporotic fractures. Although the intermittent administration of PTH is an established osteoanabolic therapy and an anti-Sclerostin antibody (Scle-AB) is currently being tested in phase 3 clinical trials, a great need exists for additional bone anabolic agents. Thus, in the context of a bi-national consortium we aim to uncover novel epigenomic networks controlling bone formation to identify new epigenomic approaches to osteoanabolic therapy. We generated tamoxifen-inducible, osteoblast-specific reporter mice (*Osx1-Cre-ERT2;dt-Tomato*) to isolate genetically labeled osteoblasts directly from bones by fluorescent-activated cell sorting (FACS). After tamoxifen injection, adult mice were treated with Scle-AB or vehicle and osteoblasts were enriched from long bones by FACS after collagenase digestion. Of all collected cells, approximately 3% were tomato-positive. Purified osteoblasts were then subjected to epigenome analyses. Therefore, chromatin was isolated followed by an analysis of a set of post-translational histone modifications, including markers of active (H3K4me1, H3K4me3, H3K27ac) and repressed (H3K27me3) genes. Our preliminary results obtained by chromatin immunoprecipitation (ChIP) showed the presence of H3K4me1 on the enhancer of Collagen 1, as well as the constitutively expressed Beta-actin (*Actb*) gene, but not on the inactive even-skipped homeobox 1 (*Evx1*) gene. While these analyses confirm the functionality of the experimental system, the ongoing genome-wide massively parallel high throughput sequencing following ChIP (ChIP-Seq) are expected to reveal novel molecular mechanisms regulating bone formation. In conclusion, we have established an *in vivo* model allowing the investigation of the osteoanabolic epigenomic landscape, which might provide a basis for the development of novel bone anabolic anti-osteoporosis therapies.

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P252

Mitochondrial DNA point mutation is associated with lower bone turnover markers

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Introduction
Mitochondrial dysfunction is associated with several clinical outcomes including diabetes and myopathy and is implicated in the human aging process. We previously showed that the mitochondrial DNA point mutation mtDNA3243A>

G is associated with lower BMD and altered bone structure. The aim of this study was to assess bone turnover markers in individuals with the mutation and controls.
Methods
We recruited 45 patients (29 female, 16 male) with the mtDNA3243A>G mutation aged 47,6±15,2 years. Cases were matched with respect to sex, age, height and menopausal status with healthy controls. All participants gave blood for analysis of general biochemistry and bone markers.
Results
Cases and controls were matched with regard to age, sex and height, but cases had a significantly lower body weight (63.6 vs 74.6 kg, $P=0.001$) and 24 of 45 patients had manifest diabetes mellitus (DM). Fasting s-CTX was measured in 36 patients and was significantly lower than in the controls (0.41 vs 0.55 µg/l, $P=0.024$). S-P1NP was measured in 39 patients and was significant lower than in the controls (45.1 vs 57.8 µg/l, $P=0.007$). The difference in P1NP but not CTX remained significant after adjusting for weight and sex. Stratifying according to DM-status, s-CTX and s-P1NP was significantly lower in cases with DM compared to their controls, whereas levels of bone turnover markers were the same in non-DM cases and controls.
Conclusion
Mitochondrial dysfunction is associated with lower bone turnover, in part possibly explained by lower body mass and diabetes. Further studies are needed to describe the effects of mitochondrial dysfunction on bone remodelling.

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P253

Investigating the osteoanabolic epigenome of aging-related bone loss in humans

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During aging bone resorption often increases while bone formation decreases, thereby reducing bone mass and bone mineral density (BMD) and leading to osteoporosis. Evidence suggests that extrinsic factors may influence bone remodeling. While poorly understood, these mechanisms may function by inducing epigenomic programs that diminish the bone forming capacity of osteoblasts. This study is part of a bi-national consortium aimed at uncovering epigenomic networks controlling the aging-related decrease in bone formation. To reach this goal, we collected human femoral heads from elderly (65–85 years) and young (25–45 years) patients undergoing hip replacement. To characterize the material, clinical parameters affecting bone metabolism (e.g. history of falls, diseases and vitamin D level) were obtained. So far, samples from 45 patients with an average age of 60 years were harvested. Interestingly, 98% had a vitamin D deficiency. Imaging of a well-defined part of the femoral head by µCT showed an approximately 10% reduction in bone mass and BMD in the elderly compared to the young group. Chromatin immunoprecipitation (ChIP) assays of intact bone tissue demonstrated a selective and specific enrichment of the active chromatin marks H3K4me3 H3K27ac on several osteoanabolic genes, including Runx2, ALP and Collagen1. Next, the sample number will be increased and all specimens will be analyzed by µCT and bone histomorphometry. Results will then be combined with clinical data and laboratory results. We will then identify epigenetic regulatory pathways controlling aging-related bone loss by analyzing gene expression (RNA-seq), genome-wide DNA methylation (MeDIP) and a selected set of post-translational histone modifications (ChIP-seq) known to control osteoblast differentiation, precursor cell fate and lineage-specific gene expression. Together, our results demonstrate the feasibility of identifying epigenetic regulation of physiologically important osteoanabolic genes from human bone samples by epigenome mapping. This may lead to the development of new drugs for the treatment of aging-related bone loss.

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P254

Genetic risk factors for knee osteoarthritis in postmenopausal ukrainian women

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Osteoarthritis is a complex pathogenesis because diverse factors interact causing a process of deterioration of the cartilage and the subchondral bone. Despite the multifactorial nature of the knee osteoarthritis, it is related to a strong genetic component. Determination of molecular genetic causes of osteoarthritis is an actual problem. There are several approaches to assess the contribution of a candidate gene in the pathogenesis of osteoarthritis. The aim of the study was to determine the alleles frequency of genes - regulators of cartilage metabolism in postmenopausal women with knee osteoarthritis in Ukrainian population, and to assess the contribution of different polymorphisms in the risk of developing the disease.
Material and methods
DNA extraction was performed using the phenol–chloroform method from whole blood. Using PCR followed by restriction digestion and visualization of the reaction products in polyacrylamide gel have been studied 77 patients with knee osteoarthritis and 125 healthy people of the same age. We studied the polymorphism 60890 A/G of vitamin D receptor gene, -764 T/G of ERα gene and -234 T/G polymorphism of collagen type 1 gene.
Results
We did not found association of polymorphism -234 T/G polymorphism of collagen type I α1 (OR = 1.55 (CI 95% 0.86–2.79)) with the risk of knee osteoarthritis developing. Also we have not found association of polymorphism -764 T/G of ERα gene (OR = 0.91 (CI 95% 0.26–3.23)) and polymorphism 60890 A/G of vitamin D receptor gene (OR = 1.21 (CI 95% 0.66–2.19)) with the risk of knee osteoarthritis.
Results
Knowing association between pathogenic alleles, candidate genes and knee osteoarthritis in Ukrainian population will allow using genetic testing to identify predisposition to the disease. The results of this study are important for a more rational organization of the prevention and treatment of the illness in the early stages of disease development.

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P255

Polymorphism of vitamin D receptor gene, estrogen receptor gene and collagen type I α1 gene for osteoporosis in Ukrainian women

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Osteoporosis has a complex etiology and is considered a multifactorial polygenic disease in which genetic determinants are modulated by hormonal, environmental, and nutritional factors.
Polymorphism of the vitamin D receptor gene has been reported to play a major role in variations for genetic regulation of bone mass but its role within various ethnic populations is not clear. Estrogens are known to play an important role in regulating bone homeostasis, they act through binding to two different estrogen receptors (ER) and different polymorphisms of these receptors were described. A polymorphism in the collagen type I α1 gene also has been associated with low bone mass and fracture incidence.
The aim of the study was to determine the alleles frequency of genes – regulators of bone metabolism in patients with osteoporosis in Ukrainian postmenopausal women, and to assess the contribution of different polymorphisms in the risk of developing the disease.
Material and methods
DNA extraction was performed using the phenol–chloroform method from whole blood. Using PCR followed by restriction digestion and visualization of the reaction products in polyacrylamide gel have been studied 180 patients with osteoporosis and 160 healthy people of the same age.
Results
We have found association of polymorphism 60890 A/G of VDD receptor gene (OR = 3.2 (CI 95% 2.2–4.6)) and -234 T/G polymorphism of collagen type type I α1 (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 ER gene (OR = 1.2 (CI 95% 0.6–2.3)).

Conclusion
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 organization of the prevention and treatment of the illness in the early stages of disease development.

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P256

OPTN and _CCDC3_ share a bidirectional promoter region that is regulated by NfkB

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OPTN seems to have an important role in bone metabolism by being part of NfkB pathway. CCDC3 is highly expressed in adipocytes and it seems to be negatively regulated by TNFa, however the mechanisms are not very clear. These genes have a 'head-to-head' orientation in the genome, which suggests that they might be regulated by a bidirectional promoter that coordinates the expression of both genes in different tissues. Using bioinformatic tools we analyzed the shared region between *OPTN* and *CCDC3* to look for NFkB putative binding sites and performed several deletion constructs to identify the most important region within the bidirectional promoter and by transfection and co-transfection assays we analyzed the activity of each construct. Our results showed that the region shared by *OPTN* and *CCDC3* can act as a bidirectional regulatory region and that NFkB can regulate both genes. We also analyzed *OPTN* and *CCDC3* expression in several mice and zebrafish tissues by qPCR and our results showed that *OPTN* and *CCDC3* seem to have differential gene expression with *OPTN* being more expressed in calcified tissues and soft tissues such as heart, eye and muscle and *CCDC3* being low expressed in these tissues and highly expressed in fat and brain. The analysis of this bidirectional promoter allows us to clarify a new level of regulation of these genes and clarify the regions that are important for the activation of each gene. Also with this work we were able to define the regions important for the NFkB regulatory effect and hypothesize a new role of OPTN and CCDC3 in bone metabolism. All of these data could be relevant to develop new therapies to control the expression levels of these genes in diseases in which there is an imbalance of *OPTN* or *CCDC3* expression.

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Comparative analysis of human and zebrafish OPTN: molecular and evolutionary perspectives

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Optineurin (OPTN) is a protein encoded by the *OPTN* gene. This protein is involved in several cellular mechanisms such as autophagy, NF-κB signaling, cellular morphogenesis, membrane and vesicle trafficking, and transcription activation. Mutations in *OPTN* have been described in glaucoma, amyotrophic lateral sclerosis and other neurological diseases. More recently, a polymorphism in this gene was also identified by a genome wide association study to be associated with Paget's disease of bone (PDB), making *OPTN* a strong candidate gene to be involved in PDB and bone; however defining the molecular mechanisms by which variants in this gene may contribute to this bone disease requires further studies. Because zebrafish has been validated as a good model to study bone related diseases, the objective of the present work was to evaluate if zebrafish could be a good system to study the molecular mechanisms through which OPTN may contribute to PDB pathogenesis. Through a comparative analysis, we observed that OPTN is encoded by a single copy gene, both in human and zebrafish, and its genomic structure is also conserved. The neighbor genes and chromosomal localization were also maintained, which strongly suggests that zebrafish *optn* is the ortholog of human *OPTN*. Bioinformatic analysis indicates that zebrafish and human *OPTN* seem to be regulated by common transcriptional factors related to bone such as TWIST, KRUEPPEL, LIM domain, NFAT, NF-κB and RXR. Furthermore, OPTN protein comparison between several species revealed a high degree of conservation in the functional domains of the protein and in its 3D structure. In conclusion, this study demonstrates that OPTN is well conserved throughout evolution, and therefore zebrafish, which has been previously validated as a good model to study bone related pathologies, could be considered to further study the biologic role of OPTN in bone diseases and bone development.

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Muscle, physical activity and bone

P258

Pamidronate may prevent muscle protein breakdown in burns by indirectly affecting cytokines

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We have shown that the bisphosphonate pamidronate (P) given to children $<$10d post-burn prevents resorptive bone loss and muscle protein breakdown. We have also shown *in vitro* that Ca modulates the inflammatory response by altering mononuclear cell chemokine production. We hypothesized that P affects muscle protein breakdown by altering cytokine or chemokine concentration directly or indirectly by lowering blood ionized (i) Ca. We retrospectively analysed biomarkers of inflammation and iCa obtained during the randomized controlled trial of P (1.5 mg/kg IV $<$10d post-burn) in children 2–18 years, all obtained in the first 100d post-admission. The relationships between each biomarker and treatment group, each biomarker and iCa were modelled as mixed ANOVA and between iCa and treatment group by mixed multiple regression, in each analysis adjusting for age at burn, body surface area burned, and time from admission, allowing for an interaction between time and group, while blocking on subject to control for repeated measures. Cytokine concentrations were log (natural base) transformed to improve approximations of normality. Time was log (base 2) transformed for better centering and interpretation. Only IL-7 was marginally different between treatment groups ($P=0.048$) and iCa was not. However, IL-4 was inversely correlated with iCa ($P=0.049$) and IL-6 and IL-7 were directly correlated with blood iCa ($P=0.036$ and $P=0.032$ respectively). IL-4 stimulates myotube differentiation from muscle satellite cells; IL-6 is associated with muscle wasting, and IL-7 may impair myotube differentiation from satellite cells. Thus in a high Ca environment such as high bone resorption, the resulting biomarker pattern is consistent with muscle wasting and failure to differentiate. Pamidronate may act in conjunction with known up-regulation of the parathyroid CaSR to lower circulating iCa and reverse these effects.

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Myths and truths on dietary supplements and nutraceuticals for musculoskeletal health: a scoping review

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Background
An inadequate intake of nutrients, low levels of physical activity, and chronic diseases contribute to reduce muscle mass and physical performance in elderly. WHO reported that number of individuals aged $\geq$60 years will triple in 2050,

with the sub-population aged >85 years that will grow faster than the others. Market of nutraceuticals and dietary supplements is growing in Italy, in particular aimed to improve health in elderly. Objective of this scoping review was to analyse the state of the art on micronutrients, available in nutraceuticals or in dietary supplements, commercialized in Italy, in order to identify, according to an evidence-based approach, which of them improve the areas typically involved in functional deterioration of the elderly: bone, skeletal muscle and nervous tissues.
Material and methods
The Italian Group for the Study of Healthy Ageing by Nutraceuticals and Dietary Supplements (HANDS) performed a scoping review through different steps: list of micronutrients available in dietary supplements and nutraceuticals, used in elderly to improve their physical functioning in three systems (bone, muscle and central nervous system); identification of relevant studies on PubMed, using as MeSH terms the selected micronutrients, adding through PubMed Search Builder the terms: 'bone', 'skeletal muscle' and 'central nervous system'; selection of effective micronutrients; identification of effective and safe dosage regimen.
Results
We evaluated 12 relevant studies (one international society guideline recommendations, one systematic review, seven randomized controlled trials, and three prospective cohort studies). Of the 39 micronutrients available in the market, only 16 resulted to have appropriate scientific evidence of their effectiveness in terms of improving musculoskeletal health in elderly: beta-alanine, calcium, creatine, fluorides, leucine, magnesium, omega-3 fatty acids, potassium, vitamin B6, vitamin B9, vitamin B12, vitamin C, vitamin D, vitamin E, vitamin K2, and zinc.
Conclusions
This scoping review shows that selected micronutrients in specific doses might effectively improve the musculoskeletal health and cognitive function in elderly. A precise analysis carried out according to the EBM principles might deliver significant benefits in the field of dietary supplements and nutraceuticals.

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P260

Effects of calcifediol versus cholecalciferol on 25(OH)D3 serum levels, appendicular muscle strength, and physical performance in post-menopausal women

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Background
Post-menopausal women generally present reduced serum levels of vitamin D, reduced VDR expression in skeletal muscle cells, and a gradual loss of muscle mass and muscle function. The relationship between serum 25-hydroxyvitamin D [25(OH)D3] levels and muscle strength has been extensively investigated, even though there is no agreement in literature. Therefore, aim of our study was to evaluate the effects of vitamin D on 25(OH)D3 levels, muscle strength, and physical performance in post-menopausal women, comparing calcifediol and cholecalciferol.
Material and methods
In this prospective study we included postmenopausal women, dividing them into two groups, according to the prescription performed (calcifediol or cholecalciferol). We evaluated at the baseline (T0) and after 6 months (T1): serum levels of 25(OH)D3, appendicular muscle strength, using the Hand Grip Strength Test (HGS) and the Knee Extensor Strength Test (KES), and physical performance, using the Short Physical Performance Battery (SPPB).

Table 1 Outcome measures assessed at the baseline and after 6 months of vitamin D supplementation.

	Calcifediol T0 ($n=103$)	Calcifediol T1 ($n=103$)	*P* values
25(OH)D3 (ng/ml)	31.74±13.03	51.80±19.85	<0.001
HGS (kg)	15.54±6.65	18.29±3.85	0.014
KES (kg)	14.49±6.92	17.05±5.62	0.050
SPPB	8.41±3.32	9.70±2.14	0.008
	Cholecalciferol T0 ($n=102$)	Cholecalciferol T1 ($n=102$)	*P* values
25(OH)D3 (ng/ml)	35.15±11.57	40.28±13.04	0.118
HGS (kg)	15,36±6.06	16.38±5.14	0.257
KES (kg)	12.12±5.74	12.58±5.67	0.627
SPPB	7.81±3.66	8.02±3.41	0.638

Results are expressed as means±S.D. Analysis was performed using a paired *t*-test.

Results
We evaluated 205 post-menopausal women, mean aged 6928±916 years, 103 treated with calcifediol and 102 with cholecalciferol. In Table 1 we showed the results.
Conclusions
Our results showed that post-menopausal women treated with calcifediol had significant improvements in serum levels of 25(OH)D3, muscle strength, and physical performance.

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P261

Bone and muscle metabolism in lung transplant recipients

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Although it is well established, that quality of life improves in patients after lung transplantation (LTx), severe musculoskeletal complications may develop. The aim of this study was to evaluate differences in the muscle markers Myostatin and Follistatin and the bone markers Dickkopf-1 (DKK1), Sclerostin (SOST) and Periostin between LuTx recipients and healthy controls. From 38 LTx patients blood samples were taken when discharged from hospital (LTx1) and 6-9 months after discharge (LTx2). Moreover, serum samples from 30 age and gender matched control subjects were obtained. Serum levels of DKK1 and of Myostatin were significantly increased (DKK1: LTX1+152.8%, LTx2+100.3%; Myostatin: LTx1+22.7%, LTx2+28.1%) and SOST levels were significantly decreased (LTx1 −33.1%, LTx2 −26.3%) in LuTx patients compared to controls. Serum levels of Periostin were significantly increased after discharge (LTx1+90.4%). In conclusion, our data give evidence for impaired muscle and bone metabolism in patients after LuTx. Elevated levels of Myostatin in LTx patients likely indicate a catabolic state of muscle metabolism. High serum levels of DKK1 correspond to reduced bone formation, whereas low SOST levels might be due to low bone mass and consequently a low number of osteocytes.

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The role of vitamin D and exercises in correction of age-related skeletal muscle changes in postmenopausal women

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The aim of the study was to evaluate the role of vitamin D and exercises in correction of age-related skeletal muscle changes in postmenopausal women.
Materials and methods
38 postmenopausal women aged 53–82 years (mean age – 67.00±7.08 yrs) were examined. The women were divided into the following groups: A – control group ($n=10$), B – women who took an individually-targeted vitamin D therapy ($n=11$), C – women who took an individually-targeted vitamin D therapy and OTAGO Exercise Programme (http://www.hfwcny.org/Tools/BroadCaster/Upload/Project13/Docs/Otago_Exercise_Programme.pdf) during 12 months. The assessment of the examined women was conducted every 3 months at the medical center. We used the following questionnaires: SARC-F, IADL-questionnaire, frailty scale, Desmond fall risk questionnaire. For evaluation of skeletal muscle function and strength, we assessed the usual gait speed and used hand dynamometry. 25(OH)D total and iPTH levels were measured by electrochemiluminescent method i.e. Elecsys 2010 analytical system (Roche Diagnostics, Germany) and test-systems cobas. The lean mass was measured by the DXA method (Prodigy, GEHC Lunar, Madison, WI, USA).
Results
At the baseline, the groups of examined women did not differ in their age, anthropometric characteristics, 25(OH)D values, data of skeletal muscle mass, strength and function. In women of the control group, the mean 25(OH)D level significantly increased after 9 months of observation (9 months – $P=0.03$) purportedly due to the seasonal factors. In women of 2nd and 3rd groups, the

25(OH)D level significantly increased after 3, 6, 9 and 12 months of observations (<0.001). The data of SARC-F, IADL-questionnaires did not change in women of 1st and 2nd groups; however, in the 3rd group the SARC-F data significantly decreased after 12 months ($P=0.02$) while the IADL data – significantly increased after 9 ($P=0.04$) and 12 months ($P=0.05$). The data of frailty scale and Desmond fall risk questionnaire did not differ in all groups during 12 months. The muscle strength significantly increased after 9 months ($P=0.01$) in women of 3rd group while in women of 1st and 2nd group this parameter did not change. The usual gait speed and lean mass assessed by DXA did not change in all groups during 12 months. The fall frequency in women of 1st group significantly increased after 12 months, in women of 2nd group it did not change while in women of 3rd group the fall frequency significantly decreased.
Conclusion
Using individually-targeted vitamin D therapy and OTAGO Exercise Programme during 12 months significantly improves daily activity, muscle strength and decreases the fall frequency in postmenopausal women.

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Vitamin D level and hand grip strength as risk factors of actual fall in patients with lumbar spinal stenosis

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Study design
Prospective study.
Objectives
The risk factors correlated with actual falls were assessed in patients with lumbar spinal stenosis.
Materials and methods
In 201 patients (M:F 65:136) with symptomatic lumbar spinal stenosis, falls history within recent one year was investigated. The factors which were expected correlation with actual falls were evaluated together. Blood chemistry was performed to evaluate serum Vitamin D level and the nutritional marker (transferrin, albumin) and hemoglobin level. Oswestry Disability Index (ODI) and EQ5D-VAS score also were investigated. Handgrip strength was measured as the muscle strength using dynamometer. And knee osteoarthritis as comorbidities were examined using the Kellgren-Lawrence Grading Scale. Falls were assessed to occur if there is more than two times within recent one year. Patients were divided two groups; Group A (Actual falls $\geqq 2$), Group B (Actual falls ≤ 1). Univariate analysis following logistic regression analysis were performed to derive risk factors with actual falls.
Results
Average age of patients was 70.7. Actual falls occurred 36 patients in total 201 patients (18.0%). In univariate analysis, serum vitamin D level, transferrin level, ODI, EQ5D-VAS, hand grip strength, and knee osteoarthritis with Kellgren-Lawrence grade scale showed statically significant differences between two groups. ($P<0.05$) For these factors, logistic regression was done to find out risk factors related with actual falls. Serum vitamin D level (P-value 0.01, odds ratio 0.89) and hand grip strength (P-value 0.003 odds ratio 0.83) were revealed as risk factors of actual falls.
Conclusion
In patients with lumbar spinal stenosis over 60 years old, serum Vitamin D level and handgrip strength are associated with actual falls. Patients with weaker handgrip strength and lower serum vitamin D level tend to fall more frequently.

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P264

UK popular sports and hip differences on bone outcomes in adolescent male athletes: The PRO-BONE study

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Sports specific training may have different impact on bone outcomes, and adolescence is a key period to optimize bone health. The aim was to examine hip differences on bone outcomes between osteogenic (football) and non-osteogenic (swimming and cycling) sports in adolescent males.

One hundred twenty one males (13.1 ± 0.1 years) volunteered to partake in this study that received ethics approval from the UK National Research Ethics Service. Participants included: 41 swimmers, 37 footballers, 29 cyclists and 14 age matched controls. Participants in the sport groups had engaged in sport-specific training for ≥ 3 hours/week for the last 3 years, while those in the control group did not engaged in these sports ≥ 3 hours/week. Bone mineral density (BMD) and bone mineral content (BMC) were determined at the hip sites using dual energy X-ray absorptiometry. Hip structural analysis software was used to obtain bone geometry parameters at the femoral neck. Results were adjusted for age, height, lean mass, calcium intake and physical activity.
Footballers had significantly higher BMD and BMC at total hip (10–23%), trochanter (13–20%), shaft (10–20%) and Ward's triangle (13–28%) sites compared to all the other groups. Footballers' BMD was also higher at femoral neck compared to the control group (19%). Footballers showed higher cross-sectional area compared to all the groups (9–19%), cross-sectional moment of inertia compared to controls (17%) and section modulus compared to cyclists (11%) and controls (21%). Hip strength index (HSI) was significantly higher in cyclists compared to controls (29%) and in footballers compared to swimmers (21%) and controls (38%). All results above were significant at $P<0.05$.
Adolescent male footballers have an enhanced osseous hip structure compared to the other groups at a clinically relevant site such as the hip. In addition, cyclists also had significantly higher HSI than controls.

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P265

Effect of pinealectomy and resistance exercise on rats tibiae morphology, mineral quantification and mechanical parameters

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The exposition of shift workers to light at night supresses the melatonin (ME) production. ME supression may contribute to the development of osteoporosis, which can be prevented and treated by resistance exercise (RE). This study evaluated the effect of ME release supression by pineal gland (pinealectomy) and the RE on rats tibiae morphology, mineral quantification and mechanical parameters. The project was approved by the local ethics committee (protocol 2014-00939). Fourty male Wistar rats were distributed in control (CNS), exercised (CNEX), pinealectomized (PNX) and pinealectomized exercised (PNXEX) groups. RE was performed in stairs during 8 weeks and the overload used was equivalent to 60% of the maximal strenght test. The trabecular microarchitecture (micro-CT), mineral quantification (DEXA) and mechanical parameters (3-point bending test) were evaluated. Anova (two-way) with Bonferroni post-test (GraphPad Prism 6.0) were used. The pinealectomy had no effect on the parameters evaluated ($P<0.05$). On the other hand, the RE increased ($P<0.05$): maximal overload (29.5% CNEX vs CNS; 34.3% PNXEX vs PNX); bone volume over total volume (%) (30.4% CNEX vs CNS; 39.8% PNXEX vs PNX); trabecular bone number (mm^{-1}) (29.7% CNEX vs CNS; 31% PNXEX vs PNX); bone mineral content (g) (27.8% CNEX vs CNS); bone mineral density (g/cm^2) (20.2% CNEX vs CNS); energy to fracture (mJ) (41.2% CNEX vs CNS); extrinsic stiffness (N/mm) (19.1% CNEX vs CNS; 32.7% PNXEX vs PNX); breaking strenght (N) (17.7% CNEX vs CNS; 13.5% PNXEX vs PNX). The RE decreased ($P<0.05$): trabecular bone pattern factor (mm^{-1}) (22.5% CNEX vs CNS); structure model index (13.6% CNEX vs CNS); trabecular bone separation (mm) 45.3% CNEX vs CNS; 41.3% PNXEX vs PNX). In conclusion, the pinealectomy had no influence on bone parameters while the RE showed to be an extremely effective non farmacological alternative to improve bone morphometry, mineral quantification and mechanical parameters in both groups.

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Inhibition of Cyclooxygenase 2 pathway modulates collagen synthesis of cytokine-stimulated fibroblasts from Ligamentum flavum

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Introduction
Hypertrophy of ligamentum flavum (LF) induces narrowing of spinal canal which develops neurogenic claudication. Several mechanisms of LF hypertrophy have

been suggested. Among them inflammatory cytokine play a crucial role in LF hypertrophy by increasing collagen synthesis. Cyclooxygenase-2 (COX-2) pathway shares inflammatory reaction from infection and arthritis. Selective COX-2 inhibitor (COX-2si) might modulates collagen synthesis via suppressing inflammatory pathway. Hence, the current study examined the effect of COX-2si in collagen synthesis of inflammatory cytokine induced LF cells.
Materials and methods
Fibroblast and LF cells were harvested and cultured. Inflammatory cytokines (IL-1, TNF-s) were utilized to stimulate collagen synthesis of LF cells. Then COX-2si was administered to stimulated fibroblasts and LF cells. Collagen synthesis, RT-PCR for COX-2 and various collagens, were performed.
Results
Fibroblasts and LF cells stimulated by inflammatory cytokines showed increase in collagen synthesis in translational and transcriptional level. Stimulated fibroblasts and LF cells with COX-2si demonstrated down-regulation of COX-2, various collagens mRNA, and finally collagen synthesis.
Summary and conclusion
In stimulated fibroblasts and LF cells, COX-2si provided therapeutic mechanism in reducing collagen synthesis. Hence COX-2si might be useful in preventing LF hypertrophy, which opens new therapeutic, preventive measures in symptomatic lumbar spinal stenosis.
Keywords: COX-2, ligamentum flavum, fibroblast, spinal stenosis
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P267

Altered bone metabolism after high fat diet and exercise: role of Wnt signaling and insulin resistance

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High fat diet (HFD), obesity, and physical inactivity characterize the modern lifestyle. This can lead to coronary heart diseases and type 2 diabetes mellitus. Recent studies have shown that these conditions frequently are associated with poor bone quality. However, the molecular mechanisms are poorly understood. To analyze the effect of HFD and exercise (EX) on bone homeostasis, we fed 6 week-old C57BL/6 mice a high fat (60% fat) or standard chow diet for 10 weeks (10-20 mice per group). Half of each group had free access to running wheels. Ten minutes before sacrifice, 3H-2-Deoxy-D-glucose (2-DG) was injected into the retro-orbital vein to investigate 2-DG uptake in tissue. Afterwards, blood, muscle and bone samples were collected. Bone mass was analyzed using micro-computed tomography, serum parameters by ELISA, and 2-DG-uptake by liquid scintillation counting.
HFD increased body weight (+14%) in sedentary mice. This increase was prevented in mice that exercised (8 km/d). Blood glucose and plasma insulin levels were increased in both HFD groups (up to +59% and +111%, respectively), but were not changed by EX. HFD decreased the uptake of 2-DG in the muscle (−55%) and in the tibial bone marrow (−44%), whereas EX increased the uptake back to control levels. The femoral trabecular and cortical bone volume per total volume (BV/TV) was reduced after HFD (−49%) while EX did not affect the BV/TV in control or HFD mice. While EX had no influence on bone turnover in control mice, it increased bone turnover in mice on a HFD (CTX: +29%, P1NP: +23%, osteocalcin: carboxylated +51%, undercarboxylated +20%). The Wnt inhibitor Dickkopf-1 was also elevated in both HFD groups and was not affected by EX.
These data suggest that Wnt signaling and insulin resistance may play key roles in the altered bone metabolism induced by HFD and a sedentary lifestyle.
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P268

Association of frailty with vitamin D in elderly women

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Objective
To investigate the relationship of frailty with vitamin D in community dwelling elderly women.
Materials and methods
A retrospective cross-sectional study was performed on women over 60 years who were not using vitamin D supplementations. Frailty status was defined using Fried's criteria: weakness, low walking speed, low physical activity, weight loss, exhaustion. Participants were classified as robust, prefrail and frail if they scored 0, 1-2, 3 points, respectively. Vitamin D (25(OH)D) concentration in serum was measured with Cobas E411. Multinomial logistic regression was used to determine the association between frailty and vitamin D.
Results
The study was performed on 161 women: 103 (64%) robust, 30 (18.6%) prefrail, 28 (17.4%) frail. In robust women group, mean age was 69.43±6.22 years and vitamin D level – 17.57±8.19 ng/ml. The youngest were robust women (mean age 69.43±6.22 years) and their vitamin D concentration (17.57±8.19 ng/ml) was the highest compared to other groups ($P=0.001$). The mean age of women in prefrail group was 70.79±7.92 years, vitamin D level – 16.17±6.38 ng/ml. The oldest women were in frail group (75.8±5.98 years) and their vitamin D level was the lowest (13.29±6.15 ng/ml). Unadjusted analysis in frailty vs robust group (reference category – robust) showed that higher levels of vitamin D were associated with being robust (OR: 0.91, 95% CI: 0.85; 0.97; $P=0.009$). After adjusting for the age, the association between frailty and vitamin D was not statistically significant ($P=0.26$). However, increasing age was found to be a risk factor of frailty (OR: 1.13, 95% CI: 1.05; 1.21; $P=0,001$). No statistically significant relationships were found in prefrailty vs robust and prefrailty vs frailty groups.
Conclusion
Unadjusted lower levels of vitamin D are associated with being frailty in elderly women, but after age adjustment the associations were not significant. No associations between vitamin D and prefrailty were found.
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P269

Effects of inorganic phosphate and FGF23 on C2C12 myoblast cells

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Background
Disturbance of systemic phosphate homeostasis is often associated with musculoskeletal dysfunction. Multiple factors related to the underlying condition such as calcium levels and endocrine mechanisms are thought to contribute. Distinct effects of inorganic phosphate itself as well as its main regulator FGF23 by activation of similar pathways have been shown in several cell types. We are not aware of any detailed investigations into their effect on the differentiation and viability of skeletal muscle cells. Therefore, we investigated their effect on skeletal muscle cells in a murine *in vitro* model.
Methods
C2C12 muscle progenitor cells were differentiated under single and combined treatments with inorganic phosphate and/or FGF23 and Klotho. Expression of differentiation markers (myogenin, MyHC, MyoD, Myf5) were analyzed by RT-PCR. Proliferation rate was analyzed by measurement of BrdU incorporation. Metabolic activity was examined by EZ4U assays.
Results
Phosphate treatments inhibited the expression of differentiation markers in C2C12 cells in a dose-dependent manner. The altered expression profile was associated with increased proliferation rates and metabolic activity. FGF23/Klotho treatments partly mimicked gene expression changes under phosphate treatment but did not alter proliferation rates.
Conclusion
High phosphate loads directly inhibited muscle cell differentiation in a C2C12 model system. FGF23/Klotho treatments partly showed similar effects. Knowledge of the distinct effects of phosphate could help us to optimize treatment of hyperphosphatemia and aid to prevent musculoskeletal diseases.
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A new tapping screw design for anterior cruciate ligament reconstruction

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Interference fit fixation of soft-tissue grafts is in clinical focus to allow anatomic graft fixation which increases stability and graft isometry. Although clinical data show promising results with different screw materials, the issue of biodegradability, handling and stability in the first weeks remain under demand. The purpose of the present study was to investigate anterior cruciate ligament (ACL) reconstruction radiologically, biomechanically and histologically using direct tendon-to-bone interference fit fixation with a newly designed tap locking screw synthesized from a mixture of ceramic and hydroxyapatite in a sheep model.
Twenty-eight skeletally mature sheep underwent a bilaterally ACL reconstruction with an autologous Achilles tendon split graft. Grafts were directly fixed with poly-(D,L-lactide) interference screws (PLLA) in 14 control sheep and with HA-screw in another 14 sheep. Animals were operated on the right knee first and left to heal for 52-weeks, however the left knee was left to heal for 6 weeks.
All grafts by time of insertion were stable and did not pullout from bone tunnel in both experimental and control group. Post mortem at 6 and 52 weeks, knees were scanned using μCt. The HA-screw showed better stability in the bone tunnel at the 6-weeks time points when compared with the PLLA screws. Also cystic legions were noticed at this time point. After 52-weeks, the HA screw bone – implant interface exhibited good osteointigration in both femoral and tibial screws. Over the whole healing period the graft fixation was not weakened through the screw design. The direct interference fit fixation withstood loads without motion restriction in all sheep.
Currently, radiological quantification, histological assessment and biomechanical testing are under investigation. However, the HA-screw provides stability in the initial 6-week critical phase.

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Skeletal muscle mass is strongly correlated with total Hip BMD in premenopausal women

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Introduction
The significance of sarcopenia and low muscle mass are especially emphasized in these days. Sarcopenia is well known to highly correlate with fragility and increases the risk of falling in the elderly and an important risk factor for disability and mortality. Evidence of inter-relaionship of muscle with bone metabolism is increasing. Therefore we investigated the relationship between BMD and muscle mass among healthy Korean premenopausal women.
Materials and methods
A total of 2,711 premenopausal women who taken DXA from 2012 and 2013 in department of health promotion center of our hospital were analyzed retrospectively using demographic data including BMI, WC, HC, waist hip ratio (WHR), muscle mass, fat mass, and lipid profile (total cholesterol, HDL, LDL, TG). Inbody measurement was used to determine fat mass and muscle mass. The Pearson's correlation coefficient (CC) was used to identify coefficiency between Z-score (BMD-L(L1-L4) and BMD-H(femur -Total) and parameters.
Results
The mean age was 44.2 years and mean BMI was $22.43 \pm 2.99(kg/m^2)$, mean fat mass and muscle mass were 18.12 ± 5.54 and 37.17 ± 3.80. WHR was 0.80 ± 0.06. Mean Z-score of BMD-L and BMD-H were 0.17 ± 0.94 and 0.33 ± 1.14. Lipid profile, fat mass, abdominal obesity and WHR did not show significant CC. Muscle mass showed a strong correlation coefficency with BMD-H (CC = 0.13, P-value $= 4.78 \times 10–11$).
Conclusions
Skeletal muscle mass is a strong correlation factor in total hip bone density among premenopausal Korean women in her forties. The importance of increasing skeletal muscle mass in young aged women should be emphasized to increase total hip BMD.

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Nutrition

P272

Obese young adults exhibit lower total and lower free serum 25-hydroxycholecalciferol in a randomized vitamin D intervention

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Objective
Although obesity is a risk factor for vitamin D insufficiency, its impact on vitamin D-binding protein (DBP) concentration, and thereby possibly also on free 25OHD, is less well known. Our aim was to compare total and free serum 25OHD, and DBP concentrations between obese and normal-weight young adults at baseline, and their responses to cholecalciferol supplementation.
Design
A 12 weeks' randomized, double-blinded clinical trial.
Patients
Obese subjects $N = 18$ (BMI = 38, 67% men) with severe childhood-onset obesity and 24 age-matched normal-weight controls (BMI = 23, 46% men), mean age 21 years. Both obese and control subjects were randomized into two groups to receive either placebo or cholecalciferol 50 μg daily.
Measurements
At baseline, 6 and 12 weeks blood samples and anthropometric measurements were collected; baseline body composition was assessed by dual-energy x-ray absorptiometry.
Results
At baseline obese subjects had, compared with controls, lower total and free serum 25OHD (49 *vs* 62 nmol/l, $P = 0.041$; 2.8 *vs* 4.7 pg/ml, $P = 0.001$), without differences in DBP concentrations (309 *vs* 346 μg/ml, $P = 0.212$). Cholecalciferol 50 μg per day increased both total and free 25OHD (ANCOVA $P < 0.001$ and $P = 0.021$). The response of total 25OHD to supplementation was inferior in the obese compared with controls ($P = 0.027$). On the contrary, the change in free 25OHD concentration was similar in groups ($P = 0.487$).
Conclusions
Obese young adults exhibit lower total and free 25OHD concentration, which is not directly explained by differences in DBP status. The response of free 25OHD to supplementation did not differ between obese and control subjects.

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P273

Food restriction harms bone properties of prepubertal, but not of young adult or elderly rats

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In this study, the effect of food restriction of 30% in calorie intake on femurs of rats of different ages was investigated. Male *Rattus novegicus albinus* rats, Holtzman lineage, aged 38 days, 4 and 16 months were used. The animals were divided into six groups ($n = 8–10$ per group), three being control groups (C38, C4 and C16) and three groups on restricted diet (R38, R4 on R16). The restricted animals were submitted to food restriction of 30% being fed with 70% of the *ad libitium* consumption of the control group. Protocol Ethics Committee on Animal Experimentation n° 057/2012. Analysis of the biometric, biophysical, biomechanical and biochemical properties of bone tissue were performed. Animals on restricted diet had a shorter femur length ($R38 = 33.39 \pm 0.73$; $R4 = 39.04 \pm 0.90$) in comparison with their controls ($C38 = 35.79 \pm 0.72$; $C4 = 40.23 \pm 0.89$), but no difference was observed between 16 months groups ($C16 = 42.15 \pm 0.94$; $R16 = 42.08 \pm 0.95$). The restriction diet reduced the thickness of the femur in the group

aged 38 days (C38 = 5.34 ± 0.41; R38 = 4.62 ± 0.18) but not in restricted to groups 4 (C4 = 6.04 ± 0.15; R4 = 5.90 ± 0.27) and 16 months (C16 = 5.81 ± 0.48; R16 = 6.06 ± 0.29). Animals aged 38 days in food restriction also presented reduction of bone volume (C38 = 0.59 ± 0.03; R38 = 0.47 ± 0.03). This effect was not observed in group 4 (C4 = 0.72 ± 0.03; R4 = 0.70 ± 0.05) and 16 months (C16 = 0.73 ± 0.40; R = 16 0.74 ± 0.04). The femoral calcium content reduced in restricted diet rats with 38 days (C38 = 134.50 ± 9.48; R38 = 88.45 ± 8.04) and 4 months (C4 = 197.10 ± 15.97; R4 = 152,00 ± 12.05), but no difference was observed for group 16 months (C16 = 170.90 ± 20.73; R16 = 164.60 ± 14.27). No differences in the biomechanical properties resulting from food restriction were observed (maximum load; maximum load until fracture; displacement until fracture; stiffness and tenacity). The diet food restriction resulted in harm to bone development with remarkable loss in prepubertal rats, inconsistent alterations in young adult rats and potential benefits to bones of elderly rats.

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Caffeine at moderate dose did not affect the skeletal system of rats with streptozotocin-induced metabolic disorders

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Diabetes leads to development of osteoporosis. Experimental type 1 diabetes may be induced by a single dose of streptozotocin (STZ), and nicotinamide (NA) administered 15 min before STZ dose-dependently protects against the STZ action. Coffee drinking, apart of its health benefits, is taken into consideration as an osteoporosis risk factor. Data from human and experimental studies on coffee and caffeine effects on the skeletal system are inconsistent. For example, although other experimental reports indicated unfavorable effects of caffeine, we previously reported beneficial skeletal effects of its moderate dose in ovariectomized rats (Mol Nutr Food Res, 2013). The present study was performed to investigate effects of moderate-dose caffeine on diabetes-induced disorders in the rat skeletal system.

Effects of caffeine (20 mg/kg p.o. daily for 4 weeks) were investigated in 3-month-old female Wistar rats ($n = 8–10$ per group), which, 2 weeks before the start of caffeine administration, were administered STZ (60 mg/kg i.p.) alone or STZ after NA (230 mg/kg i.p.). Bone mass, mineral density (BMD), histomorphometric parameters, and mechanical properties of the tibial metaphysis, femoral diaphysis and femoral neck were examined.

STZ induced diabetes, with increased bone resorption and decreased bone formation. Decreases in BMD and worsening of cancellous bone mechanical properties (the tibial metaphysis) were demonstrated. Although STZ after NA induced slight glycemia increases in first days of the experiment only, mechanical properties of cancellous bone were worsened and vertebral BMD was decreased. Administration of caffeine did not significantly affect bone histomorphometric parameters, mineralization and mechanical properties in STZ and STZ/NA-treated rats.

In conclusion, results of the present study indicate that moderate-dose caffeine did not exert damaging effect on the skeletal system of rats with streptozotocin-induced metabolic disorders. The results contribute to the notion that caffeine may not be the main culprit responsible for unfavorable skeletal effects of coffee drinking.

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P275

Vitamin D from the marine inuit diet and markers of inflammation – a population-based survey in Greenland

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Background
The traditional Inuit diet in Greenland consists mainly of fish and marine mammals. The marine diet is an important source for vitamin D in Greenland. Vitamin D has anti-inflammatory capacity but markers of inflammation were high in Inuit with a high intake of marine food items. Yet, the effect of vitamin D on inflammation in Inuit remains unsettled.
Objectives
To investigate the association between vitamin D and markers of inflammation in a population with a high intake of marine food items.
Methods
535 Inuit and non-Inuit living in West- and East Greenland participated in the survey. Interview-based food-frequency questionnaires were used to obtain information concerning dietary habits. Blood samples were drawn for analysis of 25-hydroxy-vitamin D and the inflammatory markers hsCRP and YKL-40.
Results
Participants were divided into three groups based on degree of intake of traditional Inuit diet. Markedly more Inuit than non-Inuit had a frequent intake of the traditional marine Greenlandic diet (79%), while the reverse was seen for non-Inuit, where 83% lived mainly on imported food items ($P < 0.001$). The diet groups (Inuit diet/mixed diet/imported foods) associated with vitamin D levels in serum, 74.2/69.8/52.9 nM ($P < 0.001$), hsCRP 1.6/1.4/1.3 mg/l ($P = 0.002$) and YKL-40 130/95/61 ng/ml ($P < 0.001$), respectively. A decreasing YKL-40 level was found with rising vitamin D level in Inuit (Inuit diet $P = 0.002$; mixed diet $P = 0.011$). YKL-40 level decreased with rising vitamin D level after adjusting for other factors known to influence inflammation ($P < 0.001$). However, this was not seen for hsCRP.
Conclusion
Vitamin D and markers of inflammation vary in parallel with the intake of the marine Inuit diet. Vitamin D levels were inversely associated with YKL-40 levels, but not with hsCRP. The hypothesised anti-inflammatory effect of vitamin D was not supported for the hsCRP; hence other factors in the marine diet may be speculated to influence inflammation.

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P276

Association between serum 25-hydroxyvitamin D levels and total testosterone levels in Korean adult men

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Background
There have been many studies on the association between vitamin D and testosterone in the world. But it has not been fully clarified. Furthermore, data in Korean men is limited. This study aimed to determine the association between vitamin D and testosterone levels in korean men.
Methods
This cross sectional study analyzed serum 25-hydroxyvitamin D (25(OH)D) and total testosterone in 365 Korean men over 25 years of age who visited a local university hospital. To measure the level of serum 25(OH)D and total testosterone, venous blood samples were collected from the male subjects after a 12 hours overnight fasting. The association between serum 25(OH)D and total testosterone levels was analyzed using multiple regression analysis and partial correlation analysis.
Results
The subjects were divided into four groups according to serum 25(OH)D levels. The average age was 52.45 ± 10.71 years, and the mean serum 25(OH)D and total testosterone levels were 19.42 ± 8.73 ng ml^{-1} and 5.09 ± 1.81 ng ml^{-1}. Using multiple regression analysis, after fully adjusting for several factors (age, season, body mass index, waist circumference, skeletal muscle mass, body fat, systolic blood pressure, diastolic blood pressure, hypertension, diabetes mellitus, dyslipidemia, smoker, alcohol drinking, exercise, total cholesterol, fasting plasma glucose, prostate specific antigen, HbA1c, Calcium and Phosphate), 25(OH)D is not significantly associated with testosterone. ($P = 0.084$). Also, in the partial correlation analysis 25(OH)D and total testosterone showed similar outcome after adjustments for confounders ($P = 0.05$).
Conclusion
In conclusion, we demonstrated no association between serum 25(OH)D and total testosterone.

Keywords: 25-hydroxyvitamin D, total testosterone, Korean men
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P277

Association between the sideways fall fractures and body mass index in patients from a public Mexican hospital

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The body mass index has been regarded as a risk predictor factor for fractures, and in some prospective cohort studies it has been suggested that obesity could be a protective factor for hip fractures in adults.
The present study aimed to investigate whether body mass index is related to the fracture risk when individuals had sideways falls in Mexican patients. We analyzed files from 448 patients, which checked in at Orthopedics and Traumatology Service in a public Mexican hospital. We excluded 317 files, which had recorded injuries for other causes (e.g. traffic accidents, or height higher than 2 meters). Our final sample was 131 patients who suffered fractures from sideways falls and were older than 18 years. The age mean was of 53.4 years old for women, and 39 years old for men. Statistical analysis was performed with SPSS software. Hospital Ethics and Research Committee approved the present study.
Results indicated that there was a significant association between the gender and the fracture risk ($P<0.001$), showing that women had and approximate 40% elevated risk of sideways fall fractures. The analysis (Pearson's chi square) also showed that the proportion of normal and overweight women who suffered fractures was significantly higher than underweight women ($P<0.001$) and obese women ($P<0.05$). In the case of men, normal weight, overweight and obese patients showed higher proportions when compared to underweight ($P<0.01$).
In conclusion, these results are in agreement to other reports, showing the increased risk for women. However we found that a higher body mass index does not protect from fractures in adults, at least for this sample in our region, which has one of the highest obesity rates in the country. Strategies should be designed to promote within women older than 40 years changes in lifestyle to increase the bone strength.

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P278

Association of bone mineral density, life style and diet of korean middle aged-men

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Background
Osteoporosis is the most common metabolic disease of bone and constitutes one of the most important major public health problems not only in women but also in men. This cross-sectional study examined the association of lifestyle and nutritional factors with bone mineral density (BMD) in 88 Korean middle-aged men.
Methods
Physical measurement, smoking habits, alcohol consumption, physical activity and nutritional analysis were assessed from an physical examination and interview. BMD was measured at the lumbar spine and femur neck using dual-energy X-ray absorptiometry.
Results
Patients age range from 22 to 83. Mean age, height (cm), weight (kg), BMI (kg/m^2), waist circumference (cm), calory intake of patients are 58.4 ± 12.5, 168.5 ± 5.6, 70.5 ± 10.1, 24.7 ± 3.2, 87.4 ± 5.9, 1214.9 ± 518. Height was correlated with L2, L3, mean of L-spine BMD, mean of L2 to L4 spine BMD ($r=0.468$, $P<0.05$, $r=0.413$, $P<0.05$, $r=0.399$, $P<0.05$, $r=0.421$, $P<0.05$)
Conclusion
Half of osteoporotic men have no underlying disorder, many are known to be the cause of osteoporosis. Although alcohol, smoking, low BMI, immobility, poor nutrition are known to be correlate with BMD, this study showed no relationship between them except height and some of Lumbar spine, mean of L-spine BMD, mean of L2 to L4 spine BMD.

Keywords: Men, Bone mineral density, Life style, Nutritional factors
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P279

Association between lean mass and dietary protein intake in postmenopausal women

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The aim of the study was to evaluate the appendicular lean mass depending on the dietary protein intake in the Ukrainian postmenopausal women.
Materials and methods
The study involved 63 women aged 52–89 years, who, depending on their ages, were divided into groups: 52–59 years ($n=9$), 60–69 years ($n=26$), 70–79 years ($n=21$), 80–89 years ($n=7$). To assess the dietary habits of women, we used the three-day sampling method and SEC Viria software. Lean mass was evaluated using a dual-energy X-ray absorptiometry (Prodigy, GE). We also calculated appendicular lean mass index (ALMI) by the formula: ALMI = lean mass of upper and lower extremities (kg)/height (m^2).
Results
Examination of patients' dietary habits showed an age-related decrease. Women of 80–89 years consuming <1.0 grams of protein per 1 kg of body weight accounted for more than a half of their group (57.1%), which is significantly different from the parameters established in women of 52–59 years (22.2%). For the purpose of quartile analysis, women were divided into four groups depending on their ALMI values: Q1 – ALMI = 5.20–5.84 kg/m^2 ($n=15$), Q2 – ALMI = 5.85–6.25 kg/m^2 ($n=17$), Q3 – ALMI = 6.26–6.56 kg/m^2 ($n=16$), Q4 – ALMI = 6.57–7.65 kg/m^2 ($n=15$). Women with the lowest ALMI values consume the lowest amounts of dietary protein ($F=3.67$; $P=0.02$). Significant correlations among dietary protein, nonessential, essential aminoacids and ALMI values ($r=0.40$, $t=3.44$, $P=0.001$; $r=0.39$, $t=3.30$, $P=0.002$; $r=0.35$, $t=2.91$, $P=0.005$; accordingly) were determined.
Conclusion
Further studies are needed to elaborate a set of recommendations aimed at correction of nutritional habits observed in older women of different countries.

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P280

Effects of clinically relevant doses of vitamin A on bone in mice

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Excess vitamin A has been associated with decreased cortical bone thickness and increased fracture risk. While most studies in rodents have been done using very high doses of vitamin A for a few days, we here investigate how clinically relevant doses of vitamin A, calculated from the Recommended Daily Allowance (RDA) in humans, affects the bone phenotype in mice after longer exposure.
C57BL/6 mice were fed either a control diet (15 IU/g retinyl acetate), or diets enriched with 67 IU/g (medium), or 200 IU/g (high) retinyl acetate for 4 or 10 weeks.
Serum retinol levels increased at 4 and 10 weeks with the high dose vs control, however, retinyl esters have been reported to be a more sensitive measurement of excess vitamin A, with levels over 200 nM indicating potential hypervitaminosis A in humans. Mice fed the high diet had retinyl ester levels of 343 ± 65 nM and 325 ± 96 nM at 4 and 10 weeks respectively, significantly exceeding the control of 65 ± 17 nM and 45 ± 4 nM respectively and indicating potential hypervitaminosis A. Tibial periosteal circumference decreased which resulted in reduced cortical bone area ($-11\pm2\%$; $P<0.01$) and decreased cortical thickness ($-6\pm2\%$; $P<0.05$) after 10 weeks of high diet. These reductions in the amount of cortical bone, measured by CT, resulted in a non-significant trend towards reduced bone strength as analysed by three point bending, maximal load at failure. In contrast, trabecular bone was not affected in the metaphyseal region of long bones or vertebrae as measured by CT. At 4 weeks, a decrease in the cortical bone expression of osteoblastic genes, with no effect on osteoclastic genes, was observed. This suggests that the reduction in cortical bone mass may be mediated by bone forming osteoblasts.
In conclusion, our results suggest that even clinically relevant doses of vitamin A have a negative impact on cortical bone, with the trabecular bone unaffected.

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P281

Abstract unavailable.

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P282

Waist-to-height ratio (WHtR) and coronary artery calcification

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Background
Many studies have demonstrated that waist-to-height ratio (WHtR) correlates with risk factors of coronary artery disease (CAD) better, than the body mass index (BMI). Coronary artery calcification (CAC) is an independent risk factor of atherosclerotic heart disease. However, the association between WHtR and coronary artery calcification scores (CACS) still need to be elucidated. The purpose of this study was to investigate the relationship between WHtR and CACS in healthy adults.
Method
A total of 1111 adults without histories of cardiovascular disease who visit the Health Promotion Center at the University Hospital were included in this study. All subjects were measured CACS by multi-detector computed tomography (MDCT).
Results
Participant with a CACS >0 had a greater WHtR than those with a CACS $=0$ (0.535 ± 0.006 vs 0.517 ± 0.005, $P<0.001$). After adjusting for risk factor that affect CAC, WHtR represented an independent predictor of presence of CAC (odd ratio: 1.04, $P=0.019$, 95% CI: 1.01–1.07). Male sex and systolic blood pressure associated with a 2.53- and a 1.02-fold increase in CAC, respectively ($P<0.001$, 95% CI: 1.53–4.19; $P=0.007$, 95% CI: 1.01–1.04).
Conclusion
In this study of adults without heart disease, WHtR was an independent predictor of CAC. These results suggest that WHtR may be useful marker of CAD.

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P283

The efficiency of fish scale-derived calcium supplementation on the prevention of bone loss in lactating rats

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Breastfeeding leads to marked trabecular bone loss as a result of high osteoclast- and osteocyte-mediated bone resorption. It has long been postulated that adequate dietary calcium intake prevents maternal bone loss, nevertheless, whether calcium supplement alleviates maternal bone mechanical property need to be investigated. Herein, we hypothesized that calcium supplement from natural sources could alleviate bone loss and improve mechanical property in lactating rats. During lactation day 7–21, dams were daily gavaged with water (lactating control) or elemental calcium of 16 mg/kg body weight before breastfeeding. Tested calcium products were derived from fresh-water fish scale (FS), chicken-egg shell (ES) and calcium chloride ($CaCl_2$). After the end of experiment, tibiae and femora were collected for bone mineral density (BMD) measurement and mechanical property analysis. Calcium quantification was determined by inductively coupled plasma mass spectrometry (ICP-MS) and revealed that FS and ES powders contained 434.3 ± 24.17 and 477.3 ± 4.025 mg/g, respectively. X-ray diffraction revealed calcium crystal structure in FS and ES powders were mainly carbonate hydroxyapatite and calcium carbonate, respectively. From BMD measurement by micro-computed tomography, lactating rats given FS, ES and $CaCl_2$ before breastfeeding exhibited higher trabecular BMD at proximal metaphysis. The mechanical property test by three-point bending further revealed that lactating rats given FS showed higher ultimate load, yield load and stiffness than lactating control, whereas, only ultimate load was significantly increased in those given $CaCl_2$. There was no change in mechanical property of femora of lactating rats given ES. In conclusion, fresh-water fish scale is good natural source for calcium supplementation as it can alleviate bone loss and increase mechanical property of maternal skeletons.

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P284

Is bone equally responsive to calcium and vitamin D intake from food vs supplements? Use of ^{41}Calcium tracer kinetic model

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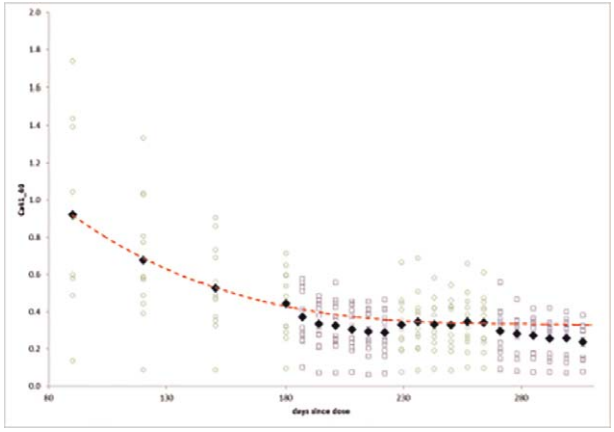
Few interventions directly compare equivalent calcium and vitamin D from dairy vs supplements on the same bone outcomes.
Objectives and Methods
Using ^{41}Ca tracer techniques, determine if 4 servings/d of dairy foods reduces Ca excretion more than an equivalent amount of Ca and vitamin D from supplements. Secondary objective was to evaluate the time course for change in Ca excretion.
Design
In this crossover trial, postmenopausal women ($n=12$) were dosed orally with 100nCi of ^{41}Ca and received dairy (4 servings/d dairy foods; ~1300 mg Ca, 400 IU vitamin D_3/d) or supplements (1200 mg Ca carbonate/d, 400 IU vitamin D_3/d) in random order. Treatments lasted 6 weeks separated by a 6 week washout (WO). Ca was extracted from weekly 24 h urine collections; accelerator mass spectrometry (AMS) was used to determine the urinary $^{41/40}$Ca ratio.
Results
Urinary $^{41/40}$Ca ratio decreased significantly with treatment, but there was no difference between treatments. The reduction in ^{41}Ca excretion was observed in 1–2 weeks ($P=0.0007$ and $P<0.001$ for dairy and supplements, respectively). Return to pre-treatment excretion levels was observed within 1–2 weeks WO ($P=0.0024$).
Conclusion
These data suggest that changes in urinary Ca excretion with increases or decreases in Ca intake occur within 1–2 weeks; most likely via the miscible pool to maintain Ca homeostasis.



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Osteoporosis: evaluation and imaging

P285

Postmenopausal osteoporosis: clinical and biological profile, about 70 cases

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Introduction
Osteoporosis is a systemic skeletal disease characterized by low bone mass and micro-architectural deterioration of bone tissue leading to an increase in bone

fragility and susceptibility to fracture. Bone is subject to the influence of exogenous and endogenous hormonal factors capable of modulating the activity of bone cells and mineralization, and also the mechanical stresses. Postmenopausal osteoporosis is the most common primitive osteoporosis. Its diagnosis requires the elimination of secondary and metabolic bone disease.
The objective of the study
Evaluate the clinical and laboratory profile, especially vitamin D, frequently low in the elderly in 70 patients followed in rheumatology for postmenopausal osteoporosis.
Materials and methods
Descriptive study conducted in patients followed in rheumatology for postmenopausal osteoporosis. Were excluded patients followed for secondary osteoporosis and those with osteoporosis densitometry discovery before menopause may sound on bone metabolism.
All patients underwent an interview and physical examination, a complete blood and urine calcium and phosphate. An analytical study was conducted and this thanks to statistical tests Student.
Results
They were 70 patients. The average age was 63 years with a standard deviation of 9.19. The average weight was 69 kg. The age of menopause was below 50 years to 75.70%, beyond the other cases. Nine fracture history were recorded. All patients were referred to bone under treatment, especially bisphosphonate orally. The median duration of prescription was 24 months. Mean serum calcium was 93 mg/l (s.d. = 5.85), serum phosphorus in 37.66 mg/l (s.d. = 5.27), urinary calcium of 24–125 mg/24 (s.d. = 34.01), 25 (OH) Vitamin D to 25 ng/ml (6.30). However, 51.3% of patients were taking concomitant vitamin D supplementation treatment. Parathyroid hormone was 45 ng/ml (12.54). Renal function was normal.
Discussion
It appears from our study, significantly advanced age compared to the definition of the disease, which states that postmenopausal osteoporosis is related to the aging population. The average weight does not reflect the obesity of our population, it does not constitute a bias on vitamin D deficiency found. Laboratory tests, usually normal in osteoporosis are made in the interest rule out other bone diseases. By comparing the results found in our study and physiological values for each analysis, it is concluded that all are in the standards. Thus, the found results are statistically significant.
Conclusion
Postmenopausal osteoporosis is the most common benign bone diseases embrittling. The Vitamin D deficiency is common and is often associated. The introduction of a target bone treatment first requires the correction of bone metabolism disorder, normal in primary osteoporosis, and research of secondary osteopathy.

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P286

Trabecular bone score (TBS) and TBS adjusted FRAX are better indicators for morphometric vertebral fractures than bone mineral density in type 2 diabetic postmenopausal women

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Type 2 diabetes is associated with fracture risk but, paradoxically, greater bone mineral density. The trabecular bone score (TBS) has been proposed as an index of bone microarchitecture associated with bone quality. This study compared the performance of TBS, bone mineral density (BMD), original and TBS adjusted FRAX scores in the prediction of vertebral fractures (VFs) in diabetic patients. This cross-sectional study enrolled 169 Korean postmenopausal diabetic women. Lateral plain radiographs of the thoracolumbar spine were taken. Lumbar spine and femur neck BMDs were obtained using dual-energy X-ray absorptiometry (DXA). TBS was obtained using the TBS iNsight Software program with BMD DXA images. VFs were diagnosed when at least one of the three height measurements was decreased by >25% compared to the nearest uncompressed vertebral body. Among the subjects, 34 women (20.1%) had VFs. Significantly lower TBS (P = 0.008) and higher TBS adjusted FRAX scores were evident for major fractures (P = 0.019) in the group with VFs compared to the group without VFs. In contrast, there were no significant differences in BMD and original FRAX scores between the two groups. Odds ratios for VFs for TBS in the lowest quartile (vs highest quartile reference) was the highest (OR 5.5, 95% CI: 1.1–27.7) compared to other parameters even after adjustment for age. These results suggest that TBS and TBS adjusted FRAX could be more useful than BMD to predict osteoporotic fractures in postmenopausal diabetic women.

Keywords: Vertebral fracture, Type 2 diabetes mellitus, Trabecular bone score, FRAX

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P287

Indentification of the lumbar 1-4 and lumbar 2-4 bone mineral density in women

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Introduction
Osteoporosis is a major bone disease among postmenopausal women. Measurement of BMD in the spine and hip is used as gold standard for the diagnosis of osteoporosis. ISCD recommended that posteroanterior L1-4 as the spine region of interest for BMD measurement. However, both L1-4 and L2-4 are used in the clinical and epidemiological studies. The aim of our study was to evaluate whether the differences of BMD between L1-4 and L2-4 would exist.
Method
We reviewed the results of spine BMD measurement by DXA from patients admitted to Our Hospital between 2013 and 2014. The bone mineral density of lumbar, (L1-4, L2-4) femoral neck and hip bones were measured in 4903 females over 44 years old. Patient consent was waived by the institutional review board of our hospital.
Results
In all groups, bone mineral density and *T*-score of lumbar vertebrae were existed significant differences $P < 0.05$. What's more, L1-4 were lower than L2-4 counterparts. With age, bone mineral density and *T*-score of lumbar showed bone mass decreased before 70 years while hip showed in all groups. The largest degree were declined in 50–55 group and there were no obvious differences between L1-4 and L2-4. After 70 years, there were some rebound in lumbar bone mineral density and *T*-score, expecially L2-4.
Conclusions
We found that skeletal sites spinal degenerative joint diseases DJD and abdominal aortic calcification have influenced the lumbar results of DXA measurement. Identifying these differences may help to diagnose osteoporosis correctly.

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P288

Underreporting of vertebral fractures with and without edema on spinal magnetic resonance imaging in adult patients

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Objective
To emphasize the clinical importance, and quantify the degree of underreporting of vertebral fractures (VF) with and without edema on spinal magnetic resonance (MR) imaging.
Methods
All spinal MR images obtained for patients ≥45 years, hospitalized from 1/6/2014 to 31/12/2015 at a single tertiary-care institution, were retrospectively reviewed to identify prevalent VFs. VF and fracture severity were classified using Genant's method. Data regarding VF diagnosis, dual energy X-ray absorptiometry (DXA) imaging, and initiation of osteoporosis treatment were extracted from each patient's MR report and medical record.
Results
Images were reviewed from 1040 patients (567 women, 55%). Mean age was 61.3 years (range: 45–93 years). 265 patients (25%) were shown to have VFs, 161 of which were single fractures (61%). VF was officially reported for only 89 patients (34%). Twenty-eight patients with reported VF also had MR evidence of bone marrow edema, however edema was documented for only half of the patients (14/28). Among 176 patients with unreported VFs, a total of 247 fractures were identified (224 mild fractures (91%), 20 moderate fractures (8%), three severe fractures (1%)). Of the 89 patients with VF, only 20 (23%) were subsequently referred for DXA, and only 49 (55%) had VF mentioned among their discharge diagnoses. Only 28 cases (31%) were prescribed pharmacologic treatment for osteoporosis (e.g. antiresorptive agents) when discharged. Of the 28 patients whose VFs were accompanied by bone marrow edema, only 6 had a subsequent DXA, and 14 were discharged on osteoporosis therapy.

Conclusion
We found that VFs are underreported on routine spinal MR imaging, especially mild fractures and fractures with edema. For reported fractures, significant gaps still exist in subsequent osteoporosis evaluation and treatment. Timely reporting of such fractures, as well as recognition of appropriate next steps in treatment should be emphasized to ensure the comprehensive care of patients.

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P289

Sleep apnea and bone mineral density

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Introduction
Obstructive sleep apnea (OSA) is a common sleep-related respiratory disorder characterized by repeated episodes of apnea and hypopnea resulting in sleep fragmentation, nocturnal hypoxia and hypercapnia, and excessive daytime sleepiness. OSA has recently become a well-recognized problem in view of its high prevalence in the general population. OSA is associated with many endocrine disorders (hypogonadotropic hypogonadism, hypercortisolism, glucose intolerance). These endocrinopathies may lead to bone loss, with secondary osteoporosis.
The aim of this study is to investigate both the bone metabolic abnormalities and bone mineral density (BMD) in OSA patients compared to individuals without OSA.
Material
The study was conducted on 25 males diagnosed with OSA and 20 healthy males. Body mass index, lean mass, and representative measures of metabolic syndrome (waist circumference, fasting plasma glucose, blood pressure, HDL-cholesterol, triglycerides) and inflammation (ESR, CRP, fibrinogen), serum calcium, phosphorus, alkaline phosphatase were evaluated. BMD in the lumbar spine (L1-L4), total hip and femoral neck was measured by dual energy X-ray absorptiometry (DXA).
Results
There were no statistical differences in age, height, weight, blood pressure, and BMI between groups. Except for the HDL-C values, significantly lower in the group with severe OSA, no significant differences in metabolic syndrome parameters were observed. Fibrinogen was significantly higher in the OSA group. Serum ESR levels were not statistically different between groups. We noted significant differences between OSA patients and control subjects with regard to lumbar L1-L4 *t*-score, lumbar L1-L4 BMD, and femoral neck BMD values ($P<0.001$). We find significant correlations with lumbar L1-L4 BMD ($P<0.05$) and lumbar L1-L4 *t*-score values ($P<0.05$).
Conclusions
OSA patients might represent a risk group with respect to loss of BMD and bone resorption. It is important to evaluate bone loss in these patients. Our study indicates that there is a relationship between OSA and osteoporosis.

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P290

Gender similarities and differences in cross-sectional cortical and trabecular bone of femoral neck in elderly Chinese population

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Objective
We aim to investigate gender similarities and differences in femoral neck (FN) structure of elderly population by bone investigational tookit (BIT) of quantitative computed tomography (QCT).
Material and methods
This cross-sectional study was part of China Action on Spine and Hip Status study, including 207 males aged 55–87 years and 400 females aged 55–96 years. QCT scans were performed in the hip for the subjects, and we used BIT software which directed automatically the lowest area of FN cross-section perpendicular to FN axis to measure cortical and trabecular bone in anatomic quadrants of FN. The measurements of cortical thickness (Cor.T), cortiacal vBMD (Cor.vBMD), trabecular vBMD (Tra.vBMD) and integral vBMD (Int.vBMD) at the FN were determined in BIT sectors. This resulted in four anatomical quadrants, Quadrant 1(Supero-anterior, SA), Quadrant 2 (Infero-anterior, IA), Quadrant 3 (Infero-posterior, IP), Quadrant 4 (Supero-posterior, SP). The study was approved by the ethics committee of Beijing Jishuitan Hospital.
Results
The greatest difference between genders after adjustment was in Cor.T of Quadrant SA, 27.3% lower in women ($P=0.000$). The differences in Cor.T of Quadrant IA and IP did not get statistical significance. There was no gender-related difference in Int.vBMD of Quadrant IA, IP and SP. However it was detected that elderly men had a higher vBMD in the trabecular bone compartment in same regions. With aging, Cor.T of superior FN declined significantly in both sexes, in contrast Cor.T of inferior region maintained in men different from that in women.
Conclusions
Our results indicate that women have thinner Cor.T in superior quadrants. With aging, Cor.T of cross-sectional FN declines significantly in women, in contrast Cor.T of Inferior region maintains in men.

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P291

Prevalence of vertebral fractures kept constant in postmenopausal women in Beijing, China: peking vertebral fracture (PK-VF) study, 2007–2012

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Purpose
PK-VF study, being conducted in 2007–2008 and 2012–2013, was aimed to investigate the prevalence and 5-year incidence of vertebral fractures (VFs) in postmenopausal women aged 50 years or older in Beijing, China.
Methods
In the 2007–2008 survey, we randomly selected a community-based population of 1825 postmenopausal women, of whom 952 women also participated in the 2012–2013 survey. Adopting the same sampling strategies as 2007–2008 survey, in the 2012–2013 survey, we additionally selected 1101 postmenopausal women, and a total of 2053 subjects were finally included. VFs were confirmed by the lateral radiographs of the thoracolumbar spine (T4-L5), being evaluated by two experienced radiologists. To investigate the secular trend of VFs, we standardized the prevalence of VFs in the 2012–2013 survey to the age composition of 2007–2008 population. Pearson χ^2 test was conducted to compare the incidence of VFs between subjects with and without VFs in 2007–2008.
Results
The actual prevalence of VFs was 24.8% (95% CI, 22.8–26.8%) in 2007–2008, and 24.1% (95% CI, 22.3–26.0%) in 2012–2013. The age-standardized prevalence of VFs in 2012–2013 was 23.3% (95% CI, 21.5–25.1%) and was insignificantly decreased 0.94-fold (95% CI, 0.84–1.05), comparing to 2007–2008. The 5-year incidence of VFs during 2007–2012 period was 5.3% (95% CI, 3.8–6.7%). Comparing to subjects without VFs in 2007–2008, subjects with VFs had a 2.605-fold (95% CI, 1.460–4.650) higher risk getting a new VF in the following 5 years.
Conclusions
The rates of VFs kept constant in postmenopausal women in Beijing, China, during 2007–2012. History of previous VF was a potential risk factor of incident VF.

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Association between plasma sphingosine 1-phophate levels and incident fractures in postmenopausal women: a 3-year follow-up observation study

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Sphingosine-1-phosphate (S1P) is a significant regulator of bone metabolism. Recently, we found that a high plasma S1P level is associated with low bone mineral density (BMD), high levels of bone resorption markers, and a high risk of prevalent vertebral fracture in postmenopausal women. We investigated the possibility that S1P is a predictor of incident fracture. A total of 263 postmenopausal women participated in this longitudinal study and received follow-up for a mean duration of 3.5 years (untreated [$n=79$] or treated with bisphosphonate or hormone replacement therapy [$n=184$]). The baseline plasma S1P level and fracture occurrence during the follow-up period were assessed. A high S1P level was significantly associated with a higher rate of prevalent fracture after adjusting for femoral neck (FN) BMD and potential confounders (odds ratio [OR] = 2.13; 95% confidence interval [95% CI] = 1.08–4.22]. Incident fractures occurred more frequently in the highest S1P tertile (T3) than in the lower 2 tertiles (T1-2) after adjusting for confounders, including baseline FN BMD, prevalent fracture, antiosteoporotic medication, annualized changes in FN BMD, and potential confounders (hazard ratio [HR] = 7.30; 95% CI = 1.05–75.75). Insufficient response to bisphosphonate therapy occurred more frequently in T3 than T1-2 (OR = 5.03; 95% CI = 1.04–24.47). The plasma S1P level may be a potential predictor of fracture occurrence and insufficient response to bisphosphonate therapy in postmenopausal women.

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DXA in clinical practice: invest in quality to improve accuracy and clinical relevance

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Introduction
Dual X-ray Absorptiometry (DXA) is currently the best technique available to evaluate bone mass, enabling the diagnosis of osteoporosis, the prediction of fracture risk and monitoring.
Clinical good practices consider as preferred measuring sites the lumbar (L1–L4) and proximal femur (neck and total). The vertebral morphometry is a quantitative method for the diagnosis of vertebral fractures based on measuring vertebral heights.
There is an uneven supply of diagnostic densitometric that historically represents a significant problem for the consequences that entails, in terms of money expenditure (duplication of incorrect examinations), of credibility of the diagnostic method and, in particular, of the accuracy of the subsequent clinical management.
Objective
Contrary to the prevailing standard in our area that includes the performance of a single scan (lumbar at age <65 years and unilateral femoral at age >65 years), the benchmark operating standard at our center, designated to the diagnosis and treatment of osteoporosis, involves running contextual lumbar scans, bilateral femoral and lateral to evaluate morphometric vertebral (VFA). We conducted a prospective data collection aimed at detecting whether there are significant differences in the two approaches considering a strictly diagnostic and clinical.
Material and methods
A total of 311 consecutive unselected patients underwent DXA examination according to the extensive method (lumbar and femoral bilateral, VFA); in each case, we have also detected what would be the diagnostic conclusion if it had applied a restrictive method (DXA lumbar age <65 years or femoral age >65 years); we also researched the impact resulting from the bilateral femoral scan, compared to unilateral; finally, we measured the impact of the VFA, in addition to DXA lumbar and femoral bilateral.
Results
In our experience, the contextual execution of the DXA in two standard sites (lumbar and femoral) produces an increase in diagnostic sensitivity of 40.5%.
The simultaneous execution of DXA in the lumbar and femoral bilateral, plus vertebral morphometry, to the execution of conventional test (vertebral or femoral) induces an increase in the percentage of pathologic findings of 47.17%.
There is a particularly significant increase in the evidence of severe osteoporosis (which is tripled, with the ability to document unrecognized vertebral deformities).
The impact of the two different approaches on clinical decisions resulted to be significant: we record a 34.4% increase of prescriptions of first-level antiresorptive therapy (alendronate, raloxifene) and more than doubled (130% increase) recourse to a second-level treatment (denosumab, zoledronic acid, teriparatide).
Conclusions
The availability of suitable equipment and the investment of resources (time and clinical skills) fully compatible and sustainable with the current practice allow us to apply operating standards in diagnostic densitometry that significantly increase the accuracy of the examination and its usefulness in practice, as well as the credibility of the diagnostic path.

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Establishing an *LRP5* mutant zebrafish (*D. Rerio*) model of bone acquisition

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Aim & background
Evidence from genetic studies of osteoporosis and fracture risk indicate that bone mineral density (BMD) is genetically controlled. The low-density-lipoprotein-related receptor 5 (*lrp5*) gene is a known co-receptor in the canonical Wnt bone formation regulatory pathway. Although mutations found in this gene have been shown to regulate BMD in humans, and *Lrp5* knockout mice had reduced bone mass, its role in the bone development/bone acquisition ambiguous. Their genetic similarity to humans, small size, and external development make zebrafish (ZF) a valuable model for vertebrate development.
Methods
We knocked out the *lrp5* gene in ZF using the CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) genome editing technology. One-cell-stage ZF embryos were injected with CRISPR-Cas9 *lrp5* guide RNA. Young adult-injected ZF were inbred to obtain F1 progeny. F1 siblings carrying mutations were then crossed to generate F2 progeny, and phenotype-genotype correlations were done using the F2 larvae. Mutation characterization was done by extracting DNA from *lrp5* mutant ZF, PCR amplification and sequencing after cloning into pGEM-T vector. Phenotyping of mutated larval ZF was done by calcein staining of vertebrae detected by fluorescent microscope. Animal experiments were approved by the Bar Ilan University IACUC (no. 52-12-2012).
Results & conclusion
ZF injected with CRISPR-Cas9 *lrp5* guide RNA were heterozygotes to *lrp5* mutations. Mutations in F1 sibling were mainly small insertions or deletions of 3-7bp, some resulting in a null allele, and their F2 progeny were mainly compound heterozygotes to *lrp5* mutations. The mutants did not differ in mineralized vertebrae number compared to WT at 8, 10 and 13 days post fertilization (dpf), which include first stages of endochondral ossification, although their body size was significantly lower at 13 dpf.
Understanding the role of *lrp5* in bone acquisition will help identify novel therapeutic targets for preventing osteoporosis.

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Risk factors for subsequent vertebral compression fracture following osteoporotic compression fracture

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Objectives
To evaluate risk factors of subsequent vertebral fracture following acute osteoporotic vertebral compression fracture.
Methods
As a multicenter retrospective study, we recruited 135 patients treated for acute osteoporotic compression fracture with available spine image taken at 1-year

follow-up in three hospitals. The patients were divided into two groups according to occurrence of subsequent vertebral fracture. Two groups were analyzed with age, sex, bone mineral density, medical comorbidity, acute fracture level, presence of prior vertebral fracture, osteoporosis medication, treatment method.
Results
The new vertebral fractures were detected in 25 patients (19%) in total. With the univariate analysis, there were no significant differences between the two groups in age, sex, medical comorbidity, presence of prior vertebral fracture and acute fracture level. However in the group with subsequent vertebral fracture, more patients were treated by vertebroplasty or kyphoplasty and had femur neck T score ≤ -2.5 and poor compliance of osteoporosis medication with significant difference ($P<0.05$). Using the multivariate analysis, it was found that subsequent vertebral fractures were significantly influenced by vertebroplasty or kyphoplasty ($P=0.003$, odds ratio$=4.71$) and femur neck T score ≤ -2.5 ($P=0.013$, odds ratio$=3.47$).
Conclusions
The subsequent vertebral fractures were found in 19% at 1 year after the treatment of acute osteoporotic compression fracture. Risk factors for them were (1) vertebroplasty or kyphoplasty and (2) femur neck T-score ≤ -2.5.

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P296

Predicting hip fracture type of elderly Asian patients with low-energy fall by volumetric BMD and femoral morphology from QCT

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Introduction
Femoral neck fractures and trochanteric fractures are two major types of hip fractures. The BMD changing or hip strength analysis (HSA) of the two type fractures may be different. No research had focused on a large sample of Asian people. This study investigated the differences in BMD and morphologic features of the two type hip fractures of elderly Asian people from QCT.
Methods
A total of 279 elderly Chinese patients with hip fractures due to low-energy fall were included (mean age, 74 years old; femoral neck fracture, $n=235$; trochanteric fracture, $n=144$). Each patient underwent QCT scan within 48 h after fracture. The femoral neck region was divided into four quadrants: inferior–anterior (IA), superior–anterior (SA), superior–posterior (SP), and inferior–posterior (IP). Cortical thickness, cortical mass fraction, cortical BMD and trabecular BMD measurement were made at each quadrant. With HSA, measurements of the mineralized bone surface cross-sectional area, the cross-sectional moment of inertia, the section modulus (Z), the buckling ratio were obtained. Linear regression equation was applied to correct coefficients as age, sex and BMI.
Results
Patients with trochanteric fractures showed higher cortical thickness, cortical mass fraction, and cortical BMD at SA quadrant than patients with femoral neck fractures ($P<0.01$; $P=0.01$; $P<0.01$). But, patients with trochanteric fractures had lower trabecular BMD at the SP, IP and IA quadrants ($P=0.02$; $P<0.01$; $P=0.03$). And, no significant differences were found in the parameters of HSA between the two groups.
Conclusions
Severer trabecular osteoporosis is seen in patients with trochanteric fractures than in patients with femoral neck fractures. However, the patients with trochanteric fractures had shown higher cortical thickness, cortical BMD, and cortical mass fraction at femoral neck superior-anterior quadrant, which may be more important to predict hip fracture type.

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Dexamethasone produces both bone-loss and bone-spare effects in $OPG^{-/-}$ mice

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The effects of dexamethasone on trabecular and cortical bone in $OPG^{-/-}$ mice were investigated in present study. Forty 6-month-old C57BL/6J female mice, including wt ($n=20$) and OPG knockout ($OPG^{-/-}$, $n=20$), were randomdly divided into four groups and treated with either sterile normal saline or dexamethasone at 1 mg/kg by intramuscular injection 3 days per week. All mice were sacrificed at 4 weeks after these treatments. Sera were harvested for biochemical analysis, while the fourth lumber vertebrae and left tibia were scanned by microCT. It was found that there was no significant difference in either CTX or P1NP in each group. In WT mice, dexamethasone did not induce any changes of bone parameters. While in $OPG^{-/-}$ mice, dramatic bone loss and micro-structural deteriorations in both lumbar trabecular and tibial cortical bone were found. However, it was also found that dexamethasone improved bone volume fraction, Tb.Th, Tb.N and decreased Tb.Sp as well as SMI in the tibial trabecuae of $OPG^{-/-}$ mice. This was firstly showed us that except for the expectedly bone-loss, dexamethasone could produce bone-spare effects in $OPG^{-/-}$ mice at specified bone sites.

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Bone mass density, yes or no: the effectiveness of BMD test in decreasing the risk for hip fractures in different demographic groups in Israel

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Background
Bone mass density (BMD) test is used for diagnosing osteoporosis and identifying patients at high risk for fractures. Hip fractures are one of the possible negative outcomes of osteoporosis. Previous studies have examined demographic variations in osteoporotic hip fractures in ethnic groups, but the predictive value of BMD for future fracture is unknown.
Objective
To determine whether a BMD test is associated with a lower risk of first hip fractures in patients aged 50 years and older in various demographic populations in Israel.
Methods
We used a retrospective study of members of Clalit Health-Services aged 50 years and older. We stratified patients by those who did and did not have a BMD during 1.1.2008.A multivariable logistic model was used to assess factors associated with having the BMD test, and propensity scores for performing the BMD test were generated. Cox proportional-hazards regression was used to examine the association between having a BMD test and the future risk of first hip fractures, in quintiles of the propensity score, up until 31/12/2014. We controlled for demographic variables and known risks factors for fractures in the model.
Results
Only in the population with the highest propensity for performing a BMD (women aged 65 and older, high socioeconomic status, Jewish ethnicity, treated with steroid medication and having osteoporosis-related diseases) there was an association between having a BMD test and a lower risk for first hip osteoporotic fracture during the 7-year follow-up, which was by 33% ($P<0.001$). Among this sub-population, the association between a BMD test and future hip fracture was the most protective among Arabs (88%, $P=0.018$), women (46%, $P=0.011$) and patients aged 65+(50%, $P<0.001$).
Conclusion
In Israel, BMD test is an effective screening tool to lower the risk for osteoporotic hip fractures mainly among high-risk sub-populations. Testing rates should be improved in marginalized populations.

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Bone geometry and microarchitecture in patients with anorexia nervosa: a possible role of mechanical loading on cortical bone structure

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Objective
To assess the effect of mechanical loading in anorexia nervosa (AN) *in vivo,* by comparing bone geometry and microarchitecture in weight bearing versus non-weight bearing bone.
Subjects and methods
A total of 26 female patients with AN, and 26 female controls matched on age and height were included. Bone geometry, volumetric bone mineral density (vBMD)

and microarchitecture were assessed using HR-pQCT (Scanco Medical, Brütisellen, Switzerland) of the distal radius and tibia. At each site, a 9.02-mm 3D axial representation comprising 110 slices with an isotropic image voxel size of 82 μm was obtained. Medical history, including history of fractures, was obtained by questionnaires. The local ethics committee approved the study.
Results
Cortical perimeter and total bone area were similar in patients and controls at both sites. Total vBMD was lower in patients with AN in tibia ($P=0.0002$) but not in radius ($P=0.11$) compared to controls. In tibia, cortical thickness (Ct.Th.) was approximately 25% decreased ($P=0.0001$) in the AN group, whereas in radius there was no significant difference. In terms of trabecular microarchitecture in tibia, all indices (bone volume/tissue volume (BV/TV); trabecular thickness (Tb.Th.), trabecular number (Tb.N) and trabecular spacing (Tb.Sp.) were impaired in AN compared to controls (P values range $=0.0000$–0.006). In radius, BV/TV and Tb.N were lower ($P=0.002$ and $P=0.0006$, respectively). Tb.Sp. was higher ($P=0.0006$), whereas Tb.Th. did not differ ($P=0.16$) compared to controls. Cortical porosity did not differ between groups at any site.
Conclusion
We found impaired bone microarchitecture in patients with AN compared to controls. The overall pattern of impairment was comparable between radius and tibia, except for Ct.th. and Tb.th. that were significantly lower in tibia, but not in radius. Thus, it is possible that these parameters are affected mainly by body weight per se, through decreased mechanical loading of the skeleton.

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P300

Bio-impedance and quantitative ultrasound to measure bone mineral density in post-menopausal women from a rural Mexican community

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It is widely accepted that the "gold" standard method for diagnosis of osteoporosis is dual energy X-ray absorptiometry, to measure bone mineral content and bone mineral density (BMD). However in the last decades other less harmful and cheaper methods were developed. Among them bioelectrical impedance analysis (BIA) is one of most commonly used and is used in analysis of body composition as well. On its side, quantitative ultrasound (QUS) is a noninvasive method of estimating bone mineral status of peripheral skeleton. In addition to bone density, QUS methods provide some structural information, which may be important in determining the fracture risk. Nonetheless, the equipment can be more expensive and require a trained technician than BIA. In this work, we aimed to compare the BMD measured by QUS and bone mass measured by BIA. The experimental design was a cohort, observational and cross-sectional study. This study was approved by the Institutional Ethics Committee. The initial sample was 117 voluntary subjects, however we applied exclusion criteria (mainly based on post- menopausal diagnosis) and the final sample was composed of 63 women. We determined the bone mass using a BIA and digital scale (Tanita© Ironman Inner Scan) and BMD using portable equipment for QUS (Sonostc© 3000).
We determined the bone mass means for each group classified according to QUS category (normal, osteopenia and osteoporosis). The results (analyzed by ANOVA, post-hoc Tukey) indicated that there were significant differences between the osteoporosis and normal groups ($P=0.012$) and between the osteoporosis and osteopenia groups ($P=0.042$), but no differences were detected between osteopenia and normal groups.
Although the sample size is small, we can conclude that BIA could be an alternative method to detect osteoporosis, being suitable to use in places, which do not have enough resources to acquire more specialized equipment.

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P301

Association of serum vitamin D with bone mineral density and breastfeeding in post-menopausal women from a Mexican rural community

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Vitamin D plays an important role for bone health, and is associated to the risk of osteoporosis development.
The aim of the present work was to determine the association between serum vitamin D concentration and the bone mineral density (BMD) in post-menopausal women living in a Mexican rural community (this study was approved by the Institutional Ethics Committee). The BMD was assessed by ultrasound and vitamin D was evaluated using a specific immunoassay. Also, all the women were interviewed to record their family history. This study was approved by the Institutional Ethics Committee.
The sample size was composed of 63 post-menopausal women, which were evaluated for weight, height, body mass index, water percentage, muscle percentage and fat percentage. In addition a blood sample was obtained for serum vitamin D levels determination. To analyze the results, we categorized the concentrations of vitamin D (high, medium, low) within each densitometry group (normal, osteopenia, and osteoporosis). Comparisons were carried out using ANOVA (post-hoc Tukey). The results showed that the normal BMD/high vitamin D subgroup had significantly higher levels of vitamin D than its counterparts (osteopenia BMD/high vitamin D, osteoporosis BMD/high vitamin D, $P=0.008$ and $P<0.001$ respectively). Also, from their family history, we compared the time women breastfed and vitamin D levels categories. The analysis showed that those women with normal BMD but low vitamin D, and osteopenic women breastfed shorter times ($P=0.008$ and $P=0.045$, respectively) than those with normal BMD and high levels of vitamin D. In conclusion, although there were not remarkable differences between BMD groups in terms of levels of vitamin D, it is interesting the relationship between breastfeeding and vitamin D levels and some other approaches must be designed to analyze the implications of this relationship.

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P302

A novel feature selection algorithm based on bone micro architecture analysis to identify osteoarthritis

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Objective
Texture information of the subchondral bone area (SBA) of 2D radiographs represents a promising possibility for evaluating the state of osteoarthritis (OA). However such features are likely to vary within the SBA and therefore the selection of the region of interest (ROI) plays a crucial role. Thus, a feature selection algorithm (FSA) is being applied in order to determine ROIs that enable an optimum discrimination between patients with and without OA.
Methods
The study included 152 standardized knee radiographs from 66 cases and 86 controls. SBA was assessed by using both fractal analysis (Bone Structure Vale – BSV) and a Shannon Entropy (SE) algorithm at predefined regions of the proximal tibia and the distal femur. The selected area of the proximal tibia involved a matrix of 3×8 ROIs, whereas a 2×2 matrix was defined for each condyle of the distal femur. SE and the BSV were calculated for each of the 32 ROIs, respectively. Based on these 64 variables, a FSA was applied to determine the variables that showed the best discrimination power.
Results
Combining the BSV and SE, the odds ratio increased significantly from 3.08 (95% CI: 1.78–5.30) to 14.82 (95% CI: 6.69–32.83) when using 15 features, and to 39.75 (95% CI: 15.41–102.51) based on ten features. By using the selected ten features the accuracy was found to be 0.86. This showed to be a significant improvement compared to the accuracy achieved when calculating a single mean value for the 3×8 ROIs of the proximal tibia alone (0.62 vs 0.86).
Conclusions
The application of a FSA in accordance with the combination of the two texture analysis methods shows a significant improvement with respect to the discrimination power between case and controls. The high odds ratios confirm that reliable results can be achieved by combining the BSV and the SE.

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P303

Heel quantitative ultrasound parameters, serum 25-hydroxyvitamin D and parathyroid hormone levels of healthy adult women in Greece

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Objective
The objective of this observational cross-sectional study is to identify heel bone properties, specifically broadband ultrasound attenuation (BUA), speed of sound (SOS) and stiffness index (SI), in healthy Greek women, as well the relation of these parameters with age, serum 25-hydroxyvitamin D (25(OH)D) and parathyroid hormone (PTH).
Material and Methods
A population of 738 community dwelling women was recruited at the health promotion events carried out by the Hellenic Society for the Support of Patients with Osteoporosis in rural and urban areas throughout Greece. PTH and 25(OH)D were measured. Heel bone properties measured using quantitative ultrasound (QUS) device. The study was approved by the Ethics Committee of Harokopio University.
Results
The descriptive data of population are shown in the table 1 as mean $\pm$ S.D. There is a significant difference of BUA ($114.05 \pm 15,7 \neq 107.66 \pm 15,7$, $P=0.039$) and SOS ($1555.67 \pm 34.26 \neq 1506.0 \pm 18$, $P=0.00$) between normal(15–65 pg/ml) and high(>65 pg/ml) PTH level respectively. SI and BUA are significantly different between all age groups as shown in table1 with $P=0.00$ for both parameters. SI is higher in 18–50 age group in relation to 51–65 and >65 age group ($P=0.00$ and $P=0.03$ respectively).
Conclusions
The mean vitamin D levels of older Greek women (>50 years) is below 20 ng/ml, as well as BUA, SOS and SI are lower in older age groups (51–65 and >65). Given that low levels are associated with increased risk for fractures, this study highlights the emerging issue of 25(OH)D insufficiency in Greek women and the need for targeted interventions.
Acknowledgements
The study was supported by the Hellenic Society for the Study of Bone Metabolism.

Table 1

	25(OH)D (ng/ml)	PTH (pg/ml)	SI	BUA	SOS
Total sample	19.87±7.57	39.67±14.75	89,95±18,38	113,78±15,67	1551,92±70,14
18–50 years	20,14±7,78	36,05±14,15	96,75±17	117,89±15,01	1560,13±88,95
51–65 years	19,36±7,66	43,29±14,85	84,51±16,7	110,12±14,6	1544,71±31,76
>65 years	17,64±6,52	47,29±16,03	75,5±16,19	104,37±16	1528,91±29

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The comparison of bone scan and MRI in osteoporotic compression fractures

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Study Design
Retrospective study.
Purpose
To estimate the usefulness of bone scan and magnetic resonance imaging (MRI) for the diagnosis of new fracture in osteoporotic vertebral fractures.
Overview of Literature
The diagnosis of new fracture in osteoporotic vertebral fractures requires simple X-ray and supplementary studies.
Methods
We analyzed 87 vertebrae in 44 patients, who diagnosed with osteoporotic vertebral fractures using bone scan and MRI within 2 months interval between August 2008 and December 2014. We compared hot uptakes in bone scan with MRI findings such as new fractures, old fractures and degenerative lesions.
Results
Hot uptakes in bone scan was matched to 48 new fractures, 26 old fractures and 13 degenerative lesions in MRI findings. It was 55% of concordance between hot uptakes in bone scan and new fractures in MRI. The rate of new vertebral fractures confirmed by MRI according to 1 level hot uptakes in bone scan was 96%, 2 levels was 50% and 3 more levels was 36%.
Conclusions
The diagnosis of new fracture in osteoporotic vertebral fractures requires simple X-ray and supplementary studies such as bone scan and MRI. We recommend more careful interpretation in multiple osteoporotic vertebral fracture patients about hot uptake lesions of bone scan.
Keywords: Osteoporotic vertebral fractures, Bone scan, MRI
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P305

The effect of cemented and uncemented implants on the measurement of proximal femur BMD by DEXA

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Objective
To test the hypothesis that the cemented and uncemented implants had effect on the measurement of proximal femur BMD by DEXA.
Methods
40 patients underwent unilateral THA using cemented ($n=20$) and uncemented ($n=20$) implants due to developmental dysplasia of the hip, femoral neck fracture or femoral head necrosis from January 2015 to July 2015 were included. Preoperative DEXA scans were acquired for the BMD of the posteroanterior lumbar spine (L1-L4) and the contralateral hip (total hip and Ward's triangle). The periprosthetic BMD of the 7 regions of interests (Gruen zones) were measured preoperatively, 1 week and 3 months after the operation by DEXA.
Results
The periprosthetic BMD of uncemented group increased 1 week after the operation, especially in Gruen zone 6($+19.5\%$, $P=0.028$) and Gruen zone 7($+17.6\%$, $P=0.054$). The periprosthetic BMD of cemented group increased in all seven zones postoperative and the maximum was 33.8%(Gruen zone 1), but there is not statistical significance in all seven zones ($P>0.05$). The periprosthetic BMD began to fall down 3 months after THA and the greatest bone loss was found in Gruen zone 7 (-31.3%, $P=0.023$). Multivariate regression analysis showed that, for Gruen zone 1, Ward's triangle BMD was a significant factor in predicting the effect of uncemeted prosthetic on periprosthetic BMD measurement ($P=0.045$).
Conclusion
BMD of proximal femur increased after the implantation of cemented and uncemented prosthetics. We recommend the periprosthetic BMD of the involved side measured 1 day before the THA as the best reference for follow-up to avoid the influence of the implants. Ward's triangle may predict the effect of uncemeted prosthetic on periprosthetic BMD measurement.
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P306

To measure or not to measure? Vitamin D and parathyroid hormone in patients with clinical risk factors for osteoporosis

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Background
Despite the large amount of studies published on the association of vitamin D deficiency with higher incidence of falls and fractures, the threshold for a sufficient serum 25(OH)D concentration remains subject to a considerable debate. There has also been no clear consensus on the assessment and treatment of vitamin D deficiency.
Objective
To examine the prevalence of vitamin D deficiency and/or insufficiency and its impact on calcium/phosphate homeostasis as well as on bone turnover in a major German cohort of individuals with defined clinical risk factors (CRF) for osteoporosis and fractures (acc. to German DVO Guideline 2009, and QFracture Score 2013).

Results
In 2014 we examined a total of 7,253 patients (mean age =62.6 yrs (s.d. 13.9); f 64.4%, m 35.6%) with CRF for osteoporosis and fractures. The prevalence of 25(OH)D serum levels <75 nmol/l was 87.7%. 25(OH)D serum levels below 50 nmol/l (deficiency) and 25 nmol/l (severe deficiency) have been detected in 55.0 and 15.7% of patients, respectively. Elevated PTH levels (>65 ng/l) have been found in 20.9% of 5,119 samples tested - with an inverse correlation to 25(OH)D serum levels ($P<0.05$) and positive relationship to increased bone turnover markers (B-AP, OC, DPD). The prevalence of secondary hyperparathyroidism (sHPT) was highest in patients with severe Vitamin D deficiency (35.3%) but common also in patients with 25(OH)D serum levels between 50 and 75 nmol/l (13.5%).
Conclusion
The high prevalence of vitamin D deficiency or insufficiency in a major cohort of patients with CRF for osteoporosis demonstrates the importance of routine measurements of 25(OH)D diagnostic and therapeutic purposes. The results put into question the approach adopted in various national guidelines which do not recommend 25(OH)D routine measurements. Additional consideration of PTH serum levels may contribute to a more adequate estimate of individual vitamin D supplementation needs.

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P307

Bone mineral density and trabecular bone score in ukrainian women with obesity

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The aim of this study was to evaluate the bone mineral density (BMD), trabecular bone score (TBS) in the Ukrainian women with obesity.
Materials and methods
1025 women aged 40–89 years (mean age – 62.7±9.7 yrs; mean height – 161.4±6.2 cm; mean weight – 73.9±13.8 kg, body mass index – 28.4±5.1 kg/m^2) were examined. The women were divided into the following groups depending on their body mass index: A – 360 women with obesity, BMI≧30 kg/m^2 (mean age – 64.0±8.9 yrs; mean body mass index – 33.9±3.5 kg/m^2), B – 665 women without obesity, BMI<30 kg/m^2 (mean age – 62.0±10.0 yrs; body mass index – 25.4±2.8 kg/m^2). BMD was measured by the DXA method (Prodigy, GEHC Lunar, Madison, WI, USA). TBS (L1-L4) was assessed by means of TBS iNsight® software installed on our DXA machine (Med-Imaps, Pessac, France).
Results
We have found that obese women have a significantly higher BMD of lumbar spine (A – 1.114±0.197 g/cm^2, B – 0.994±0.194 g/cm^2), femoral neck (A – 0.873±0.137 g/cm^2, B – 0.822±0.136 g/cm^2), total body (A – 1.123±0.108 g/cm^2, B – 1.037±0.111 g/cm^2) and ultradistal forearm (A – 0.429±0.087 g/cm^2, B – 0.371±0.082 g/cm^2) in comparison with women without obesity. When analyzing their BMD depending on age, we determined that the BMDs of lumbar spine, femoral neck and total body significantly differ in the women of age groups 40–49, 50–59, 60–69 and 70–79 yrs ($P<0.05$). At the same time, in the women aged 80–89 yrs the BMD of lumbar spine ($P=0.09$), femoral neck ($P=0.22$) and total body ($P=0.06$) did not differ significantly. The BMD of ultradistal forearm was significantly higher in the women of all age groups ($P<0.05$). TBS (L1-L4) was not significantly different in obese women compared with women without obesity in all age groups.
Conclusion
Obese women have a significantly higher BMD at all measured sites compared with women without obesity. TBS (L1-L4) did not significantly differ in the examined women.

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P308

The use of calcaneal quantitative ultrasound (QUS) combined with Asian osteoporosis self assessment tools (OSTA) in screening osteoporosis among postmenopausal women

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Background
WHO recommends diagnostic criteria of osteoporosis can be based on double energy absorption of x-ray bone densitometry (DXA), but the DXA method has some limitations such as relatively higher cost, relatively longer detection time, inconvenience in equipment transportation and so on. Quantitative Ultrasound (QUS) method has the potential to diagnose osteoporosis and it is convenient to carry around and low-cost. Asian osteoporosis self-assessment tool (OSTA) is an easy and effective way to evaluate Asian people's osteoporosis. Neither OSTA nor QUS can solely achieve the desired sensitivity and specificity when screening the osteoporosis, but it is a feasilble way to combine both methods.
Method
From September 2014 to December 2014, BMD of 118 postmenopausal women was measured in Guangzhou communities by QUS, and relative information such as their ages and BMI are required through questionnaire. Patients also went through lumbar dual-energy x-ray scans. DXA results is taken as the gold standard of osteoporosis diagnosis; by drawing ROC curve, this research evaluates the feasibility of joine use of QUS and OSTA score in osteoporosis screening, and determine the appropriate diagnosis point.
Results
When combined use of OSTA and QUS for screening, the regression curve was fitted as Y=−1.688*QUS−0.186*OSTA−3.973. Y was considered to be a predicted value. Meanwhile, the AUC of ROC draw by predicted value and DXA screening result is 0.847, SE=0.041, $P<0.0001$. AUC=0.847, SE=0.041, $P<0.0001$. When parallelly use of QUS-T and OSTA, the diagostic point set by OSTA≤-3 and QUS-T≤-2.2 can achieve youden index as 0.63, while sensitivity is 89% and specificity is 74%.
Conclusion
Quantitative Ultrasound (QUS) and OSTA score is a simple and economic method of predicting the incidence of osteoporosis among the elderly. By setting the QUS and OSTA threshold can effectively screen osteoporosis in patients at high risk.

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P309

Tbs and calcaneal ultrasonography in people with down syndrome

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Individuals with Down syndrome (DS) have a number of phenotypic features, including a short stature. It has been reported that people with DS have lower areal BMD than the general population, but this may be a biased result due to the smaller size of the skeleton, and it is unclear if individuals with DS have fragile bones. Thus, the objective of this study was to explore the skeleton of DS using two techniques, TBS and calcaneal ultrasound, which are not influenced by bone size. We included 76 persons with Down syndrome and 77 age- and sex-matched controls in the study. The quality of bone was assessed by calcaneal ultrasonography with a Sahara device (Hologic Waltham, MA). TBS was calculated from DXA scans obtained with a Hologic QDR 4500 densitometer.
The average age was 33 years (18–34). 53% were men. The parameters measured by calcaneal ultrasonography were better in people with DS than in control (QUI: 108 *vs* 93, $P=0.001$; BUA: 79 *vs* 70 dB/MHz, $P=0.048$ and SOS 1578 *vs* 1549 m/s, $P=2.1\times10^{-5}$). In the analysis stratified by sex, we found that these parameters also were higher in women with DS than in control women, while in men the differences were only significant for SOS (Table 1). TBS was similar in SD and controls (1456 *vs* 1474, $P=0.19$). In the sex-stratified analysis, differences were not found in men (1455 *vs* 1464, $P=0.68$), nor in women (1458 *vs* 1485, $P=0.13$), with more than 90% individuals having normal TBS in either group.

Table 1

	Calcaneal US	DS	Controls	*P*
♂	QUI/Stiffness	106 (29)	96 (25)	0.108
	BUA "dB/MHz"	77 (29)	73 (23)	0.538
	SOS "m/s"	1573 (46)	1552 (39)	0.037
♀	QUI/Stiffness	111 (33)	90 (17)	0.001
	BUA "dB/MHz"	80 (36)	66 (17)	0.034
	SOS "m/s"	1582 (47)	1545 (25)	8.3×10^{-5}

In conclusion, despite previous reports showing a low areal BMD in DS, this study shows that individuals with DS have a normal skeletal quality at the central and peripheral regions, as assessed by TBS and calcaneal ultrasound.

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P310

Hormone and hormone responsive stress-related molecules involved in osteoporosis in post-menopausal women

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Osteoporosis is a metabolic disorder of the bones that shows increased incidence in post-menopausal women where estrogen deficiency plays a significant role in its development. Estrogen also decreases endoplasmic reticulum stress by decreasing the expression of ER stress markers.
Using a combination of subtractive cDNA libraries and microarray analysis, a panel of estrogen dependent mRNAs/proteins was identified, including transcription factor, XBP1/1S; protein disulphide isomerase, AGR2 and heat shock protein HSP90/GP96. Not only are these proteins, either under estrogen regulation (AGR2, XBP1) or as an estrogen receptor chaperone (HSP90/GP96), but they are also associated with the ER-stress-UPR signalling pathway.
The aim of this project is to understand the interplay between reduced levels of estrogen, reduced ER stress response and the development of osteoporosis by investigating the effect of steroid hormones on mRNAs/proteins of the ER stress-UPR pathways and on their microRNAs regulators in osteosarcoma (osteoblast-like) cell lines. Identified proteins/mRNAs/miRNAs will be validated as potential biomarkers using clinical specimens from normal and osteoporosis patients.
1 nM of Estradiol in 48 hours showed a variable effect on the proliferation of osteosarcoma cell lines after stripping down 3 days using 1% in-house hormone stripped. RNAs were isolated from cultured cells and RNA libraries were constructed for sequencing, showing different genes have significantly different expression between the treated cells and non-treated controls. Commercially available qPCR arrays containing mRNAs that are associated with ER stress-UPR signalling pathways will also be used as an alternative strategy. Once identified, potential biomarkers will be validated using clinical specimens by qRT-PCR. This research will advance our knowledge and help us to understand mechanisms underlying osteoporosis. It will also generate a potential strategy to develop innovative diagnostic and therapeutic applications in the future to improve patients' healthcare.

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Increased body weight as a risk factor of intertrochanteric fracture severity in elderly women

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Purpose
Risk factors for intertrochanteric fracture (IF) severity were not studied thoroughly despite the high failure rate of osteosynthesis. The purpose of this study was to identify the risk factor for each fracture type using AO/OTA classification in elderly female patients who experienced IF.
Materials and methods
This retrospective study identified 240 women over 50 years old with an incident IF in whom dual-energy x-ray absorptiometry (DEXA) was obtained. Two independent orthopaedic specialists graded the fracture severity by AO/OTA classification. Age, body weight, height, BMI and trochanteric T-score were used in univariate and multivariate logistic regression analysis to identify risk factors for each fracture type as well as unstable IF.
Results
44.4% (106 hips) were graded as unstable IF. Lower BMD (OR 0.743, $P=0.049$), body weight (OR 0.970, $P=0.031$) and BMI (OR 0.927, $P=0.045$) predicted AO/OTA 31 A1 fracture while higher BMD (OR 1.443, $P=0.010$), body weight (OR 1.036, $P=0.010$), and BMI (OR 1.089, $P=0.023$) predicted A2 fracture after univariate logistic regression. Increased body weight (OR 1.027, $P=0.044$) was the only independent risk factor for unstable IF after multiple logistic regression.
Conclusion
The higher the body weight, the greater the likelihood of experiencing an IF that is more unstable and displaced in elderly women. Therefore, these patients should be warned about the possibility of severe form of IF and treated to lose weight.

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P312

Manganese distribution in bone tissue by SR-μXRF

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Contemporary elemental imaging techniques are greatly contributing into the bone research. Synchrotron radiation induced confocal micro x-ray fluorescence technique (SR-μXRF) was employed for the analysis, being the most powerful and sufficient tool in detection and characterization of trace element distributions in bone tissue. Manganese (Mn), as a potential contributor into the mechanisms of calcium incorporation into bone tissue is in the spotlight of research. Further it was demonstrated in studies of red deer antlers that mechanical competence of antler was associated with Mn content in antler material. Lower Mn content was found to result also in a lower work to peak force. Thus it is of important medical interest, if osteoporotic fractures are also correlated with Mn content.
Red deer antlers were used as a suitable, easily accessible experimental model for the studies of bones. As antlers grow fast without undergoing remodelling, it is possible to observe the direct effect of dietary regimen and environmental conditions such as Mn uptake. As Mn to calcium (Ca) correlation might give more insight into pathogenetic mechanisms of osteoporosis, analysis of samples of human bone tissue (osteoporotic and non-osteoporotic) was performed. Human and animal bone samples were obtained in compliance with applicable ethical requirements. Elemental maps of Mn and Ca were obtained; the data were subsequently processed to gain the Mn to Ca countrate ratios, which allows comparing Mn spatial distribution in antlers and human bone samples. In antlers increased Mn content (200x) in the outer rim in contrast to adjacent bone tissue was observed. Human bone samples (transiliac bone biopsy samples) from male patients with idiopathic osteoporosis tend to contain less Mn globally in bone tissue as compared to healthy controls. In bone samples taken from femoral neck of osteoporotic women such difference in Mn content compared to controls could not be demonstrated.

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Changes of trabecular bone score in Asian females: comparison of 1990s and 2010s

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The purpose of this study is to understand the changes of TBS scores in Asian females by comparing 1990's and 2010's DXA images.
We have compared the DXA images of 3774 Korean females (age: 20–79) to Japanese (age: 20–79). While Korean DXA images were obtained in 2014 and 2015, Japanese data were obtained in 1996. Participants who have abnormal DXA images or lumbar disease were excluded. TBSiNsight® software (v. 2.1.1, Med-Imaps, Boedeaux, France) was used to calculate the TBS as well as to obtain BMD values from DXA images.
Korean showed higher values of height and weight than Japanese in all age groups. So, they showed similar BMI which are 22.2 kg/m^2 for Koreans and 22.9 kg/m^2 for Japanese. However, Korean showed higher TBS than Japanese in all age group. On average, TBS of Koreans are 0.281 higher than that of Japanese and the difference is bigger in the elderly.
There is a significant difference in the slope and breaking points of TBS values between Korean and Japanese. Japanese showed drastic reduction of TBS in 21.6 years but only 15.3 years in Korean data. Also, we have observed the higher TBS values in all age groups of Korean population which might be explained by the time-difference of data acquisition (1990's vs 2010's) as well as different nutrient.

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Inducing osteoporosis in a large animal model: influence of ovariectomy, diet and corticoid therapy

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Purpose
The aim of our study is a micro-CT-based, longitudinal characterisation of ovariectomy, multideficient diet and corticoid therapy impact on the ossal microarchitecture.
Materials and methods
Four randomized groups were formed out of 32 adult female merino land sheep:
1. control
2. ovariectomized (OVX)
3. OVX + multideficient diet (OVX-D)
4. OVX-D + 320 mg methylprednisolonacetat every 14 days (OVX-D-S)
Biopsies of the iliac crest were taken initially, after 3 and after 8 months. Micro-CT-based examinations of the biopsies using an isotropic voxel size of 7 µm regarding bone mineral density (BMD), relative bone volume (BV/TV), trabecular thickness (Tb.Th.), trabecular number (Tb.N.), trabecular separation (Tb.Sp.) and structural model index (SMI) were taken.
Results
The data showed no sign of osteoporosis in the control-group, in the OVX- and OVX-D group.
However, a significant change in bone mineralisation and bone architecture in the OVX-D-S group could be shown after 3 and 8 months. BMD (231.4 ± 45.9 mg/cm^3 vs 167.59 ± 25.8 mg/cm^3 vs 135.7 ± 26.7 mg/cm^3; $P < 0.05$), BV/TV ($21.2 \pm 3.8\%$ vs $13.7 \pm 1.8\%$ vs $12.9 \pm 2.1\%$; $P < 0.05$), Tb.Th. (143 ± 13 µm vs 101 ± 11 µm vs 88 ± 6 µm; $P < 0.05$) and Tb.Sp. (581 ± 82 µm vs 631 ± 82 µm vs 627 ± 91 µm; $P < 0.05$).
With regard to Tb.N. and SMI no significant changes in the OVX-D-S group could be shown.
Conclusion
When comparing the different groups, significant changes could be found regarding BMD and microarchitecture by combining OVX, diet and methyl-prednisolone. This may be an interesting model for preclinical osteoporosis research, especially with regard to the development of bone substitute materials.

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MRI analysis of the spine in 17 adults with WNT1 osteoporosis

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Objectives
A heterozygous missense mutation p. C218G in *WNT1* was recently identified as the cause of severe primary osteoporosis (Laine *et al*., New engl J Med 2013). The mutation has thus far been identified in two large Finnish families presenting with dominantly inherited, early-onset osteoporosis, with affected adult patients showing reduced bone mineral density (BMD), vertebral compression fractures, kyphosis and height loss. This study examined characteristics of WNT1 osteoporosis in the axial skeleton using magnetic resonance imaging (MRI). The study was approved by the Research Ethics Committee.
Methods
This study included 17 adults (12 females) with a heterozygous p. C218G *WNT1* mutation. MRI scans were taken of the axial skeleton with a focus from Th5 to L5. All scans were reviewed independently by an experienced radiologist and an orthopedic surgeon. Images were assessed for vertebral morphology and signal intensity, vertebral endplates, vertebral corner defects, intervertebral disc shape and intensity, spinal canal width as well as overall spinal stature and scoliosis.
Results
The 17 subjects ranged in age from 11 to 76 years (median 49 years). Overall the MRI scans showed several changes, especially in the thoracic spine; mildly to severely collapsed vertebrae (in 9/17 subjects), elongation of vertebral shape (in 4/17), and defects in vertebral endplates (in 10/17). Intervertebral discs were abnormally high with degenerative signal loss. All changes were more common in older subjects; thoracic kyphosis was evident already in young adults and 7/8 of those over 50 years had vertebral compression fractures.
Conclusions
This study confirms that the mutated WNT1 alters thoracic vertebral shape and intervertebral discs, causes changes to endplates and predisposes to vertebral collapse and compression fractures, particularly in thoracic spine. Changes are apparent already in young adults and gradually increase with age resulting in severe spinal pathology. Children are rarely affected.

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Correlations between trabecular bone score (TBS), sex, age, height, weight and bone mineral density (BMD): a study of the Korean population

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The purpose of this study is to assess the correlation of trabecular bone score (TBS) to variable factors, i.e. sex, age, body size and BMD. Moreover, we want to find variables of correlation coefficient change with aging.
We randomly selected 2,906 subjects (1726 female and 1180 male, age range 20's to 70's) from several hospitals in Korea. Participants who have abnormal Dual Energy X-ray Absorptiometry (DXA) images or lumbar disease were excluded. TBSiNsight software (v. 2.1.1, Med-Imaps, Boedeaux, France) was used to calculate the TBS score as well as BMD values from DXA images. Statistical analysis was performed using correlation analysis. Among female participants, we have compared TBS and BMD values of those who are older than 65 years with all age group.
The correlation coefficient of TBS and BMD was the highest in 30's and gradually decreased with aging. Correlation coefficients between TBS and age ($R = -0.68$), height ($R = 0.54$), LS-BMD ($R = 0.72$) in all female participant were significantly higher than male but R values were decreased in the age group above 65.
In this study, TBS showed a strong correlation with age, BMD in both gender. However, TBS showed a significant correlation with height in female participants only. TBS and BMD showed high correlation but gradually decreased with aging. Therefore, we believe TBS and BMD have different mechanism of accessing bone quality in aged group. In conclusion, TBS should be used together with BMD for osteoporosis diagnosis especially in elderly.

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Cellular and extracellular investigations of healing parameters in a sheep model of osteoporosis

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Due to its huge socio-economic impact a better understating of osteoporotic fracture healing is crucial.
Thirty-one female merino land sheep were randomly divided into four groups. (i) Untreated control-group (C, $n = 8$); (ii) bilateral ovariectomy (OVX, $n = 7$); (iii) OVX and calcium-deficient diet (OVXD, $n = 8$); and (iv) OVXD and additional biweekly corticosteroid injections (OVXDS, $n = 8$). Drill-hole defects (7.5 mm in diameter) were created in the iliac crest. Healing time points were 5 months (M) and 8 M post fracture. Bone healing was histomorphometrically assessed using Movat Pentachrom staining. Cellular changes were detected using TRAP and ALP staining, immunhistochemistry of Type I Collagen (Col I) was

performed. Image J was used for image analysis and SPSS software was used to explore statically significant results.
Histomorphometry showed higher total ossified tissue (TOT) and lower total cartilage tissue (TCT) in control group from 5 M to 8 M marking the healing process. In contrast, OVXDS group showed the highest TCT and the lowest TOT at 8 M. Osteoid formation was lower after 8 M than 5 M in control and OVX group, in OVXD and OVXDS group osteoid was higher. The total number of osteoclasts was lower after 8 M than after 5 M in each group. Ratio of osteoclast resorption pits to bone surface was significant lower in control and OVX group throughout the time points compared to OVXD and OVXDS. ALP positive area was lower after 8 M than 5 M in all experimental groups. Interestingly OVXDS group showed significantly higher levels of ALP than all other groups at both time points. Col I signal intensity and area was higher in C and surprisingly in OVXDS group after 8 M compared to 5 M, OVX and OVXD showed lower values after 8 M compared to 5 M.
Currently Col X, RANKL and ASMA IHC are being explored for further cellular discrepancies in osteoporotic fractures.

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Circulating microRNA in metabolic bone diseases-osteoporosis

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Osteoporosis is the commonest worldwide age-related bone disease. It is clinically symptomless until the first fracture happens. Circulating microRNAs can be used as novel biomarkers to assess health status and progression of complex diseases. A recent review highlighted the involvement of microRNAs in the control of bone formation and remodeling. Most of these studies have been done on animal models, but few on human blood samples.
This research aims to identify circulatory microRNAs associated with osteoporosis in a test group of patients using advanced PCR arrays. The identified potential biomarker microRNAs will be validated using individual clinical specimens in single samples.
Ethical approvals (REC: 11/NW/0593/NHS R&D 4195/UoL000760) prior to patient recruitment were obtained. Patient blood samples were pooled into four groups: osteopenia, osteopenia with fracture, osteoporosis and osteoporosis with fracture. They were subjected to RNA extraction using QIAGEN miRNeasy kits and miRNA expression profiling was performed using the Qiagen Human Serum & Plasma 384HC miRNA PCR Array kit, with data analysis carried out using Qiagen software.
We investigated the expression of microRNAs in serum pools from osteopenia and osteoporosis patient groups. A panel of 49 up or down differentially expressed miRNAs (by >3 fold) between osteopenia and osteoporosis patient groups identified. MiRNAs expression on selected numbers of individual clinical samples were performed using 26 differentially expressed miRNAs by qRT-PCR, and seven of them showed a significant difference (by >2 fold) between the two groups.
Small RNA can be successfully isolated and identified from serum and plasma from people with osteoporosis disease and differential occurrence is evident. Future work is aimed at validating identified up or down differentially expressed miRNAs in different cohort of clinical samples; to understand the role of the identified differentially expressed miRNAs in pathogenesis and to assess whether they can be used as a biomarker for osteoporosis.

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P319

CORTEX; catching osteoporosis on routine tomography as an added EXtra

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Risk factors for osteoporosis are often present among patients referred for diagnostic CT scans. Recent UK Clinical Guidelines (NICE-CG146) promote the initial use of FRAX in the majority of patients at risk of osteoporotic fracture, in any health setting. We gained approval and NHS Innovation funding for a pilot service whereby patients undergoing a routine clinical CT scan of the abdomen or pelvis could be screened by estimating their 10-year fracture risk. Patient and radiographer inconvenience were negligible; the short WHO FRAX questionnaire was simply made available for patients to fill in while waiting for their CT scan. We collected the FRAX questionnaires weekly. In those with moderate/high risk, CT scans were automatically retrieved from the PACS archive for asynchronously-calibrated Mindways BMD analysis, performed on diagnostic CT image sequences already taken. Analysis and reporting was performed semi-automatically by a part-time NHS technician remote from the CT department. Following the analysis, full BMD results and recalculated FRAX + BMD were automatically associated with the CT scan images in the electronic imaging archive (PACS) + medical notes (eMR), along with pre-specified treatment recommendations generated from 'standard phrases'.
We audited the service against NICE CG146. Our technician was available for 42 days during the pilot during which 119 females aged ≥ 65 handed in FRAX questionnaires. They had an eligible contrast-free scan type covering their hips and/or spine, no bone-active medication and no prior DXA. A total of 86 women were at risk (72.3%), of whom 69 had satisfactory imaging to permit spine and/or hip bone density estimation. Treatment for osteoporosis was recommended for 11.6% of those patients (eight women); while 88.4% could be reassured (61 women). The CORTEX pathway is simple and feasible without interrupting clinical CT services. We consider that a trial of cost and clinical effectiveness of CORTEX in preventing fragility fracture is warranted.

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P320

Age and sex features of femoral geometry in patients with hip fractures

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According to the literature data, some parameters of the femur (length of axis, length of femoral neck) are independent predictors of hip fractures, but such studies among Ukrainian patients are absent. The purpose of this study was to study age and gender features of some geometric parameters of the upper third of the hip in patients with intra- and extra-articular fractures of the femur.
One hundred and seventy-four survey radiographs of the hip joints of patients aged 50–89 years (median age 70.98 ± 0.99 years) were analyzed, 154 of whom (97 women and 77 men) were hospitalized with intra- and extra-articular hip fractures. Assessment of geometry parameters of the femur was performed on the contralateral limb in relation to fracture. For the analysis, patients were divided into subgroups by gender, age and the localization of fracture.
It was established the significantly lower rates of cervical intertrochanteric distance (71.56 ± 2.19 and 65.36 ± 4.76 mm, $t=4.74$, $P=0.04$) and neck angle (131.00 ± 0.71 and $128.09 \pm 1.45°$, $t=4.18$, $P=0.05$) in women compared to men without fracture. In addition, significant effect of age on femoral geometry parameters in men and women with intra- and extra-articular fractures was found, but not in patients without fractures.
We found the significant correlation between age and length of hip axis ($P=0.0002$), length of femoral neck ($P=0.00006$), intertrochanteric distance ($P=0.001$), basis of the head ($P=0.0004$) and head diameter ($P=0.005$) in men with intra-articular fractures and significant correlation between age and length of hip axis ($P=0.03$), length of femoral neck ($P=0.0008$), intertrochanteric distance ($P=0.03$), head diameter ($P=0.005$), horizontal offset ($P=0.008$) and cortical thickness ($P=0.00005$) in men with extra-articular fractures of the femur. But we did not find the significant differences of hip parameters in women.
Identified differences should be considered for both planning surgery after hip fracture and for predicting the risk of hip fracture in older age patients.

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P321

The *T*-score standard in osteoporotic sheep model: insight into independent reference group

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FDA guidelines recommend the sheep as large animal model for osteoporosis. Studies of osteoporosis induction use dual energy x-ray absorptiometry (DEXA)

to evaluate bone status. Experimentally, the *T*-score is calculated based on reference bone mineral density (BMD) values of animals before treatment (0 M). Whereas clinically the reference BMD represents values of an independent group of patients around 30 years old. The study hypothesize that the use of additional independent reference group will increase stringency of the acquired *T*-score.
Thirty-one female merino land sheep with an average age of 5.5 years were used for osteoporosis induction. Following groups were compared: (i) control-group (C; $n=8$); (ii) ovariectomized group (OVX; $n=7$); (iii) OVX combined with a deficient diet (OVXD; $n=8$); (iv) OVXD combined with methylprednisolone administration (OVXDS; $n=8$). Further, an independent group of 32 healthy sheep (4–6 years old) were taken as a reference group. BMD was measured at 0 M, 3 M and 8 M. BMD of clinically relevant regions; femur head and lumbar vertebrae (LV) were calculated.
Initially peak bone mass was tested for all animals pre-treatment. Fluctuation in BMD values peaked twice at 5 years and at 9 years of age. However, the BMD drop was dramatic after 9 years. The T score depending on the 0 M as reference reflected a lower score in the OVXDS at 3 M and 8 M (-3 and -4.2, respectively). However, utilizing the independent reference group, the *T*-score of the OVXD declined into -1.8 at 3 M and -2.8 at 8 M.
Triple therapy has successfully reduced the BMD of sheep model. The use of an independent reference group changed the interpretation of the data; thusly calculated osteoporotic bone status at 3M in OVXDS group became osteopenic. Internal reference might falsify *T*-score results, and is more suitable for *Z*-score calculation. Therefore, a collective online database can enhance experimental *T*-score calculation.

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P322

The essential role of bone biochemistry in the treatment of osteoporosis with rPTH therapy: a large study of over 450 patients

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The effect of recombinant parathyroid hormone therapy as an anabolic agent has delivered great success in the treatment of osteoporosis. Although a treatment response is still defined in DXA terms, initial scans can fail to detect an increase in BMD since the collagen deposited in bone may not yet have mineralized. It is key therefore that an early response is detected biochemically and the physician can be reassured that the bone is not adynamic, the patient is compliant and in the majority of cases a DXA response will follow.
Methods
Four hundred and sixty patients who were commenced on recombinant PTH attending the bone health clinic had bone biochemistry performed at baseline and at follow up 3, 6 months and yearly there after. Bone Biochemistry included PINP (procollagen type 1 amino terminal peptide), osteocalcin (OC) and C telopeptide (Ctx).
Results
Eighty-five percent of the patients were female, mean age 65.5 ($\pm$11.15 S.D.) and males 66.1 ($\pm$9.4 S.D.). By 1 year significant changes had been made in Lumbar spine BMDs over baseline ($P<0.01$) similarly at the total hip changes were almost significant ($P=0.055$). Baseline PINP (35.1$\pm$21 ng/ml), OC (20.1$\pm$10ng/ml) and CTx (0.12$\pm$0.02 ng/ml) However marked increased in PINP were noted at 3 months ($P<0.001$), as in Osteocalcin ($P<0.01$) without a significant change was noted in serum CTx or calcium. Less that 5% of patients did not demonstrate a biochemical response. A small number who did demonstrate a bone marker rise failed to consequently demonstrate a BMD rise.
Conclusions
Bone biochemistry provides detailed information on specific activities relating to the osteoblasts ability to form bone and allows the physician to pin point potential difficulties with therapeutic interventions. They robustly indicate an early treatment response and correlate well with DXA results. They identify non responders early and those who have mineral deposition problems.

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P323

Trabecular bone score (TBS) and body composition analysis in liver transplantation patients at risk of new-onset diabetes (NODAT)

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Background
Previous TBS studies in type 2 diabetes have suggested a deterioration of bone microarquitecture that could be related to a higher risk of fractures. However, there are no information in liver transplantation (LT) patients with new-onset diabetes after transplantation (NODAT).
Our aim was to investigate TBS in LT patients and the influence of NODAT in these patients. Also, we investigated the relationship between TBS and body composition parameters.
Methods
Sixty nine LT patients (age 57.9$\pm$10.5 years) were studied. BMI and waist perimeter were obtained in all patients. A 75 g oral glucose tolerance test was performed in non diabetic and ADA diagnostic criteria were followed. Lumbar and femoral BMD were measured with DXA densitometer (QDR 4500, Hologic, USA). TBS was calculated from DXA exam (TBS iNsight 2.0 software, Med-Imaps, Switzerland). Body composition parameters included total body mineral content (BMC), total fat mass (FM), total fat free mass (FFM) and percentage of fat mass (%FM).
Results
Thirty six percent of patients had impaired glucose tolerance (IGT), and 42% fulfilled NODAT criteria. TBS showed positive correlations with lumbar BMD ($r=0.37$, $P<0.01$), femoral neck BMD ($r=0.31$, $P=0.01$) total femoral BMD ($r=0.25$, $P<0.05$) and total BMC ($r=0.32$, $P<0.01$). An inverse correlation was found between TBS and BMI ($r=-0.32$, $P<0.01$) and FM ($r=-0.26$, $P<0.05$). We found statistical differences in waist perimeter and %FM between patients with normal tolerance, IGT and NODAT ($P<0.05$). However, no significant differences were found in TBS, lumbar or femoral BMD, BMC, FM or FFM.
Conclusion
In LT patients, TBS is related to fat mass parameters and BMD. With this technique, we have not found deterioration of bone microstructure in patients with NODAT.

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Osteoporosis: pathophysiology and epidemiology

P324

Serum 25-hydroxyvitamin D, parathyroid hormone, and bone mineral density in Korean perimenopausal women

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Introduction
This study aims to assess 25-hydroxyvitamin D—25(OH)D—status in perimenopausal women to analyze its relationships with serum PTH levels and bone mineral density (BMD).
Methods
A total of 102 individuals who visited to climacteric clinic in Sanggye paik hospital participated in the study. Serum 25(OH)D and intact parathyroid hormone (PTH) were measured. BMD was determined by dual x-ray absorptiometry (DXA) at lumbar spine, femoral neck, and total hip.
Results
Serum 25(OH)D levels were below 20 ng/ml in 73% of participants. There was a significant seasonal difference in mean serum 25(OH)D, with higher levels in summer–autumn. In Pearson correlation, serum 25(OH)D was negatively correlated with PTH ($r=-0.219$, $P=0.03$), not with the age and BMD. In ANOVA, BMD was significantly low in the women older than 60 years ($P=0.000$). Level of 25(OH)D and PTH was not different among different age groups. No difference was shown in level of PTH and BMD among different 25(OH)D groups.
Conclusions
Vitamin D insufficiency is very common among in Korean perimenopausal women. Serum 25(OH)D is negatively correlated with PTH ($r=-0.219$, $P=0.03$). BMD can decrease with age, although the level of 25(OH)D was not decreased.

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P325

Bone mineral density of lumbar spine and femur in patients with gynaecologic cancer

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Objective
To compare the bone mineral density (BMD) of the lumbar spine and femur in postmenopausal women with cervical and endometrial cancer without bone metastasis with that in normal control postmenopausal women.
Methods
We retrospectively analysed the BMD of the lumbar spine and femur using dual-energy X-ray absorptiometry in 130 patients with cervical cancer, 68 patients with endometrial cancer, and 225 healthy controls.
Results
The serum levels of calcium, phosphorus, osteocalcin, and total alkaline phosphatase, and urine deoxypyridinoline were measured in all participants. Age, BMI, parity, and time since menopause were not significantly different between the three groups. The *T*-scores of basal.
BMD at the fourth lumbar vertebra (L4) were significantly lower in patients with cervical cancer (-0.68 ± 0.10) compared to those in the other two groups. Additionally, the incidence of osteoporosis at L4 according to the basal status of bone mass was significantly higher in patients with cervical cancer (10.0%) compared to that in controls (0.4%). Urine deoxypyridinoline levels were significantly higher in patients with cervical cancer compared to those in controls. No differences in basal BMD of the lumbar spine and femur were observed between patients with endometrial cancer and controls, and no significant differences in biochemical markers were detected between patients with endometrial cancer and controls.
Conclusion
Our results suggest that postmenopausal women with cervical cancer have a lower BMD and are at increased risk of osteoporosis in the lumbar spine before receiving anticancer treatment compared with postmenopausal women with endometrial cancer.

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P326

Calcium and vitamin D supplementations: 2015 position statement of the Korean society for bone and mineral research

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Calcium and vitamin D are essential components for bone health, thus calcium and vitamin D supplementation is an important strategy in the management of osteoporosis.
However, the benefit of calcium and vitamin D supplementation on bone health is still controversial. Moreover, potentially harmful effects of excessive calcium supplementation on cardiovascular health are recently suggested. Too high a level of vitamin D has been also reported to have several, possibly related, harmful events. Korea is well known for low dietary calcium intake and vitamin D deficiency in its population. This position statement developed the following recommendation for adequate levels of calcium and vitamin D intake in Korean, postmenopausal women and men older than 50 years: Adequate calcium intake and optimal vitamin D level are essential for preventing and treating osteoporosis in postmenopausal women and men older than 50 years. We recommend a daily calcium intake of 800–1000 mg/day. Food remains the best source of calcium; however calcium supplements should be considered when dietary intake of calcium is inadequate. We recommend dietary vitamin D intake of more than 800 IU per day, a level which appears to reduce the risk of fractures. When vitamin D deficiency is suspected, serum 25-hydroxy-vitamin D (25-[OH]D) level should be tested. We suggest that a serum 25-(OH)D level greater than 20 ng/ml is generally appropriate for prevention of osteoporosis. However, a serum 25-(OH)D level greater than 30 ng/ml is probably helpful for management of osteoporosis and prevention of fractures.

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P327

Gene expression profiling of osteoblastic cells cultured with lithocholic acid or bilirubin. Implications in the pathogenesis of osteoporosis in liver diseases

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Low bone formation is considered to be the main feature in osteoporosis associated with cholestatic and end-stage liver diseases. Previous studies have demonstrated the deleterious consequences of retained substances such as lithocholic acid (LCA) and bilirubin (Bil) on osteoblastic cells. These effects are neutralized by ursodeoxycholic acid (UDCA). To gain new insights into cholestatic-induced osteoporosis, we have assessed the differential gene expression of osteoblastic cells under varied culture conditions.
The experiments were performed in human osteosarcoma cells (Saos-2), cultured with LCA (10 μM), Bil (50 μM) or UDCA (10/100 μM) at 2 and 24 h. Expression of several signalling pathways and related bone genes were assessed by TaqMan microfluidic cards. The 88 genes covered a broad range of functional activities including apoptosis, osteoclast and osteoblast differentiation and mineralization and expression of genes involved in collagen synthesis and degradation, growth factors and vascularisation.
LCA up-regulated several genes, most of them involved in apoptosis (BAX, BCL10, BCL2L13, BCL2L14) but also MGP, BGLAP, SPP1 and CYP24A1, and down-regulated BMP3, BMP4 and DKK1. Parallel effects were observed by Bil, which up-regulated apoptotic genes and CSF2, and down-regulated antiapoptotic genes BCL2 and BCL2L1. Moreover, Bil down-regulated BMP3, BMP4, DPP1 and SMAD6. UDCA has specific consequences since differential expression was observed particularly at 100 μM up-regulating BMP2, BMP4, BMP7, CALCR, SPOCK3, SPP1 and DMP1, and down-regulating antiapoptotic genes and RANKL. UDCA down-regulated collagen genes with no changes in metalloproteinases. Furthermore, most of the differential expression changes induced by LCA and Bil were partially or completely neutralized by UDCA. No differential expression effects of LCA and Bil were observed regarding metalloproteinases, MAPKs, growth factors, vascularisation and oncogenes.
These observations reveal novel target genes, whose regulation by retained substances of cholestasis may provide new approaches for understanding the pathogenesis of osteoporosis in cholestatic and end-stage liver diseases.

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P328

Use of proton pump inhibitors and hip re-fractures among Austrian hip fracture patients

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We retrospectively analyzed the incidence of hip re-fractures in Austrian hip fracture patients devoid of anti-osteoporotic drug treatment, taking vs not taking proton pump inhibitors (PPIs).
For 31,668 patients ≥ 50 years sustaining a hip fracture in Austria between July 2008 and December 2010, information on hip re-fractures with follow-up until June 2011 and on filed prescriptions of PPIs and anti-osteoporotic drugs between July 2007 and June 2011 was available. A total of 17,228 patients on PPIs not receiving anti-osteoporotic drugs like bisphosphonates were identified and categorized into five sub-groups: (1) PPIs began before or (2) after first fracture, (3) PPIs discontinued before first fracture or (4) began no sooner than 30 days after discharge from first fracture, and (5) PPIs taken only short-term in hospital and/or within 30 days after discharge from first fracture. Each subgroup was sex- and age-matched with 4568 control hip fracture patients receiving neither PPIs nor anti-osteoporotic drugs. Re-fractures were related to followed-up survival years and compared statistically.
Elevated numbers of hip re-fractures/1000 patient years (py) were associated with PPI medication vs control when PPIs were begun before (28.6 vs 16.7, $P < 0.0001$), after (26.0 vs 16.8, $P < 0.0001$), and not until 30 days after first fracture (33.6 vs 16.7, $P < 0.0001$). By contrast, PPI use before first fracture only and under exclusively peri-operative short-term PPI use entailed fewer hip re-fractures per 1000 py among female patients relative to controls (7.7 vs 20.2, $P < 0.05$, and 8.4 vs 22.5, $P < 0.05$, respectively).
It is elusive whether elevated numbers of hip re-fractures among PPI users are causally linked to PPI effects or reflect greater comorbidity. Reduced re-fractures among patients on PPIs exclusively before hip fracture are likely due to few survival years, i.e. high mortality. This is not the case for short-term peri-operative PPI use, therefore not affecting or even lowering hip re-fracture incidence.

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P329

The role of melatonin in regulating autophagy in type 2 diabetic osteoporosis

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Objective
The study of melatonin on the levels of autophagy in bone will contribute to new type 2 diabetes osteoporosis-pathological processes by which a specific role and mechanism for the treatment of type 2 diabetes-induced osteoporosis could emerge.
Method
This study consists of a two-part experiment. We assessed different body parameters in rats, a type 2 diabetes model application, utilizing different concentrations of melatonin interventions, the application of dynamic biomechanical measurements, bone organization hard slice dyeing, and micro-CT, which are all methods for rat bone micro-structure observation. We also applied immunohistochemistry method of rat bone organization within autophagy level observation. We also performed *in vitro* experiments on hFOB1.19 cells cultured with high glucose, different concentrations of melatonin and ERK pathway inhibitors and then performed western blotting and immunofluorescence on cells to assess their osteogenic ability and levels of autophagy.
Results
(1) Melatonin could significantly improve the bone microstructure of our rat model of type 2 diabetes and reduce the levels of autophagy. 100 mg/kg is better than 50 mg/kg. (2) Melatonin could improve the osteogenic capacity of osteoblasts at high glucose levels and reduce the levels of autophagy in osteoblasts cultured at high glucose levels. 10 μM is better than 1 mM.
Conclusion
The proper concentration of melatonin can inhibit the ERK signalling pathway and thus reduce the levels of autophagy in osteoblasts as well as delay type 2 diabetes-induced osteoporosis.

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P330

Regression analysis between multiple osteoporotic spine fracture and osteoporotic fracture risk factors

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Objectives
There are some studies that evaluated changes of bone marrow density (BMD) and re-fracture after zoledronic acid injection. However, there is no study that showed about the fracture on all of the body, fracture healing, and improvement of clinical symptom after zoledronic acid injection. In this study, authors evaluated the 1-year BMD change, changes of lumbar pain in lumbar spine fractures, effects on fracture healing, re-fracture and additional fracture on other site after zoledronic acid injection in patients with spine and non-spine fracture.
Methods
Patients who had lower than -2.5 (T-score) at BMD from January 2011 to June 2012 in our hospital were evaluated. Among them, patients who had spine fracture and/or non-spine fracture were enrolled. Zoledronic acid was injected in 3 days after fracture. BMD was checked 1 year later after injection. Furthermore, changes of lumbar pain in lumbar spine fractures was evaluated every weeks, effects on fracture healing, re-fracture and additional fracture on other site also were evaluated for this study.
Results
Spine fracture group ($N=97$) and non-spine fracture group ($N=31$) showed significant increasing of BMD ($P<0.05$). Visual analogue scale for lumbar pain after spine fracture was reduced from 6.6 ± 2.2 before zoledronic acid injection to 4.2 ± 1.1 after injection. There was no delayed union or non-union after non-spine fractures. However, new fractures developed in three cases (2.34%); two spine fractures, one distal radius fracture.
Conclusions
There are increasing of BMD, relief of lumbar pain, no disruption to fracture healing after injection of zoledronic acid for osteoporotic patients who has spine fracture or non-spine fracture is good treatment option.

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P331

The effects of vitamin D and sarcopenia on bone mineral density in Korean women

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A osteoporotic fracture has become a global health issue that causes tremendous impact on mortality as well as heavy socioeconomic burden. Previous studies suggested that vitamin D may prevent fractures by improving muscle mass as well as via increasing bone density directly. The purpose of the study is to determine that the influence of vitamin D on bone mineral density depends on its effects on muscle mass.
We analyzed the data from Korean National Health and Nutritional Survey IV in 2009. Women older than age 20 were included for the analyses. Bone mineral density and muscle mass were measured by DXA. Serum vitamin D concentration was tested.
Vitamin D and muscle mass affected BMD at proximal femur, but not at lumbar spine. Vitamin D deficiency and sarcopenia increased odd ratio for osteoporosis before and after adjusted for multiple variables. The effects of vitamin D deficiency on BMD still remained significant after adjustment for sarcopenia, which was vice versa.
Though vitamin D deficiency and sarcopenia shared common effects on BMD, they have their own effects on BMD independent from each other.

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P332

Elevated body iron stores and circulating osteoprotegerin as independent predictors of hip fracture in postmenopausal women admitted for fragility fracture: time for new screening strategies?

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Background
Identification of risk factors may help us to understand the pathogenesis of osteoporotic hip fracture as well as to formulate development of better diagnostic, prevention and treatment strategies. The present study was designed to determine the impact of multiple metabolic risk factors such as markers of systemic inflammation (C-reactive protein), body iron stores (ferritin), insulin resistance (HOMA-IR) and bone remodeling (osteoprotegerin), for the prediction of hip fractures in postmenopausal osteoporotic women.
Methods
The study group consisted of 115 postmenopausal women divided into two groups: Group 1 consisted of 49 subjects hospitalized in the Orthopedic Department, Wolfson Medical Center due to the diagnosis of non traumatic hip fracture and Group 2 contained 66 postmenopausal osteoporotic women without history of hip fracture. Metabolic parameters were determined.
Results
Circulating OPG was significantly higher in Group 1 than in Group 2 (205.2 ± 177.1 vs $60.0=\pm22.3$, $P<0.0001$). While levels of hemoglobin as well as MCV and MCH did not differ between groups, circulating ferritin was significantly higher in Group 1 than in Group 2 (217.9 ± 195.1 vs 49.7 ± 31.3, $P<0.0001$). In multiple linear regression analysis, which explains about 40% of the variability in CRP, 42% in OPG, and 28% in ferritin, significant by-group differences in terms of these parameters persisted even after adjustment.
Conclusions
Elevated body iron stores reflected by serum ferritin concentrations and bone remodeling marker, osteoprotegerin, are independent predictors of hip fracture in postmenopausal women hospitalized for fragility fracture.
Keywords: osteoprotegerin; ferritin; hip fracture; postmenopausal women; iron

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P333

Risk factor profile and bone mass findings

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Objective
To evaluate prevalent risk factors, medication use and comorbidities with bone mass findings at the total hip, femoral neck and lumbar spine.

Design
Historical cross-sectional study.
Methods
A total of 6285 women and 565 men consecutively referred to an osteoporosis specialist clinic from 2000 to 2012 were included. Information of potential risk factors was obtained from a clinical database. Additional information on medication use, comorbidities and fractures was obtained from national registries. The study was approved according to national legislation. Patient information was anonymized. Statistics were performed using generalized linear models.
Results
A total of 212 (37.5%) men and 1857 (29.6%) women had a *T*-score below −2.5 at one of the measured sites. In women increasing age, lower BMI, increasing glucocorticoid use within one year, prevalent chronic pulmonary disease, low daily dietary consumption of calcium, current smoking, use of calcium supplementation, low exercise level, former osteoporosis treatment, prevalent major osteoporotic fractures, estrogen deficiency, prevalent rheumatoid arthritis, having an alcohol related diagnosis, use of loop diuretics, prevalent hyperthyroidism and having a family history of fractures were all found to be independently associated ($P<0.05$) with lower bone mass at either one site. Thiazide treatment was consistently associated with higher *T*-scores. In men some risk factors could not be evaluated due to low prevalence. Increasing age, low BMI, prevalent MOF, former osteoporosis treatment, low exercise level, high Charlson score, prevalent hyponatremia, having limitations in everyday life and a familial fracture disposition were predictors ($P<0.05$) of low bone mass at one or the other site. Glucocorticoid use and increasing age were associated with higher BMD at the lumbar spine.
Conclusions
Risk factors attributable to hereditary causes, life style and comorbidity were identified. Exercise level, serum sodium status, use of loop diuretics and prevalent chronic pulmonary disease, risk factors not included in fracture risk calculators were associated with low bone mass.

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P334

Incidence of clinical vertebral fractures in north-eastern Germany

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Background
Current studies concerning the incidence of vertebral fractures are rare. Knowledge about age and sex dependency of these fractures could help focussing therapeutic decisions. Therefore, we performed a prospective study in order to find a reliable incidence of clinical vertebral fractures in an adult population.
Methods
Included were all patients with radiologic confirmed clinical vertebral fractures, which occurred in the adult population (age ≥ 20 years) of Rostock, a city in the north eastern part of Germany (173.839 adult inhabitants). The time of observation was April 2014 until March 2015. All medical institutions of the city were involved. Clinical symptoms (backpain, trauma) were radiological adjudicated at the same time. Only fractures of the thoracic and the lumbar spine were analysed.
Results
A total number of 108,1/100.000 new vertebral fractures was found. Women (133,8/100.000) developed more fractures than men (80,8/100.000).
The mean age at the time of fracture was 73.4 ± 13.0 years. On average, female patients were 6 years older than men ($P<0.05$).
In female patients aged 50–59 years the incidence was 17.9, one decade later 68.2 ($P<0.05$), the highest number was found in the age group 80–89 years: 712.7. Under the age of 59 years men had significant more fractures than women ($P<0.05$).
Osteoporosis was known only in 48.9%. The most frequent causes of fractures were falls (50%). The same percentage of fractures occurred spontaneously without osteoporosis.
Discussion
There is only one comparable prospective study in Europe (EPOS), whose incidences were nearly eight times as high as our results. EPOS observed a selected population of voluntary participants. A new fracture was defined by comparing radiography after periods of time.
In contrast to EPOS our study involved cases, which were confirmed as true clinical vertebral fractures and we observed the whole unselected adult population of Rostock.
Conclusion
Female gender and high age are associated with high fracture incidence. Falls are relevant risk factors. Focussing on these risk factors may improve treatment results.

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P335

Qulaity of life of patients with osteoporotic vertebral compression fractures

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Introduction
As Korean society have been entered to aging population, osteoporosis is considered one of the most important disease in the elderly. Osteoporotic vertebral compression fractures can affect to patient's quality of life. But, in Korea, there is few studies about this, so we investigated.
Aim
To evaluate the quality of life of patients with vertebral compression fracture.
Methods
This study was performed in multicenter, prospectively, using interview. Study population was over 50 years old patients who had osteoporotic vertebral compression fracture recently and treated for the fracture. We investigated demographic features and information about vertebral fracture, such as timing of diagnosis, level of fracture, treatment methods. And satisfaction level, VAS pain scale, EQ-5D scale, Oswestry Disability Index were also recorded from population. Statistical analysis was used, and a factors were analyzed which related to quality of life.
Results
Total 304 patients were included, average period from fracture to participation of interview was 9.4 months. Multilevel fracture patients were 106. About 30% of patients were not received bone mineral density examination, and osteoporotic treatment. Mean *t*-score of bone mineral density was −2.75 ± 0.7. About 40% of patients did not have trauma history or clear memory of trauma. EQ-5D index was significantly influenced by gender and age, and significantly correlated with ODI score (correlation coefficient 0.825). Multivarient analysis revealed that multi-level involvement, bone mineral density were correlated significantly with EQ-5D index.
Conclusion
Patients with OVCFs had very low quality of life despite of fracture healing. EQ-5D is adequate tool of evaluate quality of life of osteoporotic vertebral compression fracture patients in Korea. EQ-5D index has significant relationship with ODI score. And, multilevel involvement and bone mineral density can be a factor which affects to quality of life.

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P336

Epidemiology of community-dwelling elderly vertebral fracture in China southern city: study of osteoporotic fractures (Part I)

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Objective
Osteoporotic fracture is the most severe complication of osteoporosis and vertebral column suffer the highest risk. The aim of the part I study was to evaluate the prevalence of vertebral fracture, distribution of prevalent fractures as well the grade of the fractures in community-dwelling elderly in China southern city.
Methods
A population of 6142 elderly over 50 years old from four communities in China southern city was gathered voluntarily through chester sampling from July 2014 to October 2015. All participants took X-ray plain film of lateral thoracic and lumbar spine by Rontgen unit in community health centre and the films were read by an experienced radiography observer in local People's Hospital. Semiquantitative assessment by Genant *et al*. was adapted in grading the fracture and grade1 deformity or higher was considered fractured.
Results
Till December 2015, 4197 reports were prepared and analysed consisted of 1898 male (50–94 years) and 2299 female (50–90 years). The prevalence of vertebral fracture is 7.17% for male only and 11.05% for female only, among which, 137 happened at thoracic spine only, 158 took place at lumbar spine only and 95 individuals had fractures at both places. Of those 390, L2 is the most prevalent as 17.95% and T12 had the highest prevalence in all thoracic body which is 13.85%. The table shows prevalence for each age group.

P years	60～64	65～69	70～74	75～79	80～84	85～
Male (n=1898)	2.56%	6.38%	6.35%	6.14%	11.02%	13.85%
Female (n=2299)	7.69%	6.39%	11.56%	14.52%	17.20%	28.38%

Conclusions

This is the first and pioneering study focused on vertebral fracture of community-dwelling elderly in China. The prevalence of vertebral fracture in China southern city is 9.29% in total. Women are more risky than man at any age group. T12 and L2 is the most prevalent in thoracic and lumbar spine respectively. The whole study will be a longitudinal epidemiology research and more relationships with other diseases as hypertension and diabetes will be investigated in following parts.

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P337

Longitudinal increase in vitamin D binding protein levels after initiation of tenofovir/lamivudine/efavirenz therapy among HIV-infected individuals

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Tenofovir disoproxil fumarate (TDF) is a critical component of first-line antiretroviral regimens for HIV worldwide. However, TDF-containing regimens have been associated with decreased bone mineral density and increased fracture risk, which may in part be mediated through secondary elevations in parathyroid hormone (PTH). Prior cross-sectional data suggest vitamin D binding protein (DBP) levels may increase with TDF exposure leading to a functional vitamin D deficiency, which could explain the increase in PTH.

We performed a secondary analysis using plasma samples collected at 0, 24, and 48 weeks after initiation of TDF/3TC/EFV from 134 adult participants enrolled in a multi-center randomized trial. Data regarding socio-demographic characteristics, body mass index, $CD4^+$ counts, and HIV viral load were obtained as part of the parent study. Laboratory analyses included DBP, intact PTH (iPTH), total 25-hydroxyvitamin D (25OHD), phosphorus, and markers of bone resorption and formation. Repeated measures ANOVA was used to measure change in biomarkers over time.

Our sample included 108 men and 26 women (mean age 33.6±9.6 years). Median $CD4^+$ count increased significantly from baseline to 48 weeks [290.5(201–362) vs 424(294–555) cells/mm^3, $P<0.001$], and median viral load decreased from 53767 (IQR: 19802–136493) to 0 (IQR:0–10) copies/ml. Median levels of DBP increased significantly from baseline to 48 weeks [154(91.8–257.4) vs 198.3(119.6–351.9) μg/ml, $P<0.001$]. A concurrent rise in iPTH levels was observed over the same period [32.3(24.4–40.9) vs 45.2(35.1–60.4) pg/ml, $P<0.001$), however 25OHD and phosphorus levels remained stable. Bone resorption and formation markers increased rapidly from 0 to 24 weeks, followed by a slight decline or plateau, but remained significantly elevated at 48 weeks ($P<0.001$).

Our study provides longitudinal data supporting a potential role for DBP in bone loss associated with TDF-based therapy. Further research to elucidate the mechanistic pathways and clinical impact of these findings is warranted.

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P338

Nonalcoholic fatty liver disease is associated with postmenopausal osteoporosis

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Osteoporosis is known to be associated with metabolic diseases characterized by insulin resistance, such as central obesity, diabetes, and metabolic syndrome. Non-alcoholic fatty liver disease (NAFLD) is also increased in such insulin resistant conditions. However, little is known about whether osteoporosis and nonalcoholic fatty liver disease are etiologically related to each other or not. We investigated whether bone mineral density(BMD) is associated with NAFLD in pre- and postmenopausal women. Five hundred eighty-one female subjects (266 premenopausal and 315 postmenopausal) were enrolled. Lumbar BMD was measured using dual-energy X-ray absorptiometry. Liver ultrasonography was performed to check the severity of fatty liver. We excluded subjects with a secondary cause of liver disease. Blood pressure, lipid profile, fasting plasma glucose, alanine aminotransferase (ALT), aspartate aminotransferase, and body mass index were measured in every subject. Mean lumbar BMD was lower in subjects with NAFLD than those without NAFLD in postmenopausal women (0.91±0.01 vs 1.02±0.02 g/cm^2, $P=0.041$). Multiple correlation analysis revealed a significant association between mean lumbar BMD and NAFLD in postmenopausal subjects after adjusting for age, body mass index, ALT, smoking status, and alcohol consumption (β coefficient −0.063, 95% CI −0.102 to −0.029, $P=0.001$). Even after adjusting the presence of metabolic syndrome, the significance was maintained (β coefficient −0.045, 95% CI −0.092 to −0.007, $P=0.028$). Lumbar BMD is related with NAFLD in postmenopausal females. We suggest that postmenopausal women with NAFLD may have a higher risk of osteoporosis than those without.

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P339

High prevalence of reduced bone mineral density and undertreatment of osteoporosis in patients with systemic sclerosis

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Purpose

Systemic sclerosis (SSc) is a rare inflammatory rheumatic disease that has been associated with an increased risk of low bone mineral density (BMD). However, data on risk factors associated with bone loss in SSc are scarce. The objective of this study was to investigate the prevalence of and the risk factors for low BMD in patients with SSc.

Methods

Cross-sectional data of 61 patients with SSc were collected. BMD in the lumbar spine, total hip and femoral neck was assessed using DXA. Osteoporosis and osteopenia were defined according to WHO definitions. SSc disease severity was defined by the Rodnan skin score and the Medsger disease severity score. Analysis for factors associated with BMD was performed through multiple linear regression analyses.

Results

Patients were on average 56.7±12.4 years old and 72% were female of whom 77% postmenopausal. BMD measurements revealed osteopenia or osteoporosis in at least one site in 67% of the patients, of whom 30% had osteoporosis. Low BMI and postmenopausal state were significantly associated with low BMD in all skeletal sites. No significant associations were found between age, disease severity, glucocorticoid treatment and BMD. Of the 30% of patients who had an indication for anti-osteoporosis treatment, 91% did not receive anti-osteoporosis medication.

Conclusions

A high frequency of osteoporosis or osteopenia (67%) was demonstrated in our patients with SSc. Low BMI and postmenopausal state were identified as the most important risk factors. No relationship between disease severity and BMD was found, which could be related to the sample size, although it can be argued that low BMI, at least partly, is a marker of disease severity. This study also shows a high frequency of undertreatment of osteoporosis in this patient group. These results underline the importance of monitoring and treatment of low BMD in SSc.

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P340

Current trends and future projection of hip fracture in South Korea using nationwide claims data

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Introduction
The purposes of this study were to evaluate the trends in the incidence and mortality of hip fracture between 2008 and 2012, and predict the number of hip fractures in Korea up to 2025, using nationwide claims data.
Methods
Nationwide claims data managed by the National Health Insurance Service (NHIS) was used to identify patients with hip fracture. All new visits or admissions to medical institutes for hip fracture aged 50 years or more between 2008 and 2012 were included. The incidence and mortality of hip fracture were calculated using Poisson distribution from 2008 to 2012. Projections of the number of hip fractures were conducted using Poisson distribution on the historical incidence with population projection from 2016 to 2025 in Korea.
Results
The incidence of hip fractures (per 100,000) increased by 14.1% over the 5 years of the study. The incidence of hip fractures increased 15.8% in women and 10.9% in men. A steep rise and shift in the incidence of hip fracture was distributed in the older age group from 2008 to 2012. The cumulative mortality rates at first year after hip fractures were 17.2% (3575/20,849) in 2008 and 16.0% (4547/28,426) in 2012. Overall average SMRs of hip fracture was the higher in men (11.93) than women (11.22). The SMRs were observed to be higher than those of general population in all age periods. From 2016, the total number of hip fractures are estimated to have an overall increase of 1.4-fold for the 10 year projection in 2025.
Conclusions
The incidence of hip fracture still continue to increase and the mortality after hip fracture is also high even though the mortality has decreased as time goes by. Nationwide strategies should include attention to prevent the burden of hip fracture and reduce the future socioeconomic burden.

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P341

Trend of incidence, mortality, and future projection of vertebral fracture in Korea using nationwide claims data

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Introduction
Vertebral fracture has been recognized as a major health concern. Our purposes were to evaluate the trends in the incidence and mortality of vertebral fracture between 2008 and 2012 and predict the number of vertebral fracture that will occur in Korea up to 2025, using nationwide data from the National Health Insurance Service (NHIS).
Method
A nationwide data set was evaluated to identify all new visits to medical institutes for vertebral fracture in men and women aged 50 years or older between 2008 and 2012. The incidence, mortality rates and Estimates of the number of vertebral fracture were calculated using Poisson regression.
Result
The number of vertebral fractures increased over the time span studied. Men and women experienced 14,808 and 55,164 vertebral fractures in 2008 and 22,739 and 79,903 in 2012, respectively. This reflects an increase in the incidence of vertebral fracture for both genders (men, 245.3/100,000 in 2008 and 312.5/100,000 in 2012; women, 780.6/100,000 in 2008 and 953.4/100,000 in 2012). The cumulative mortality rate in the first year after vertebral fracture decreased from 8.51% (5955/69,972) in 2008 to 7.0% (7187/102,642) in 2012. The overall standardized mortality ratio (SMR) of vertebral fracture at 1 year post-fracture was higher in men (7.76, 95% CI: 7.63–7.89) than in women (4.70, 95% CI: 4.63–4.76). The total number of vertebral fractures is expected to reach 157,706 in 2025.
Conclusion
The incidence of vertebral fracture increased in Korea in the last 5 years, and the socioeconomic burden of vertebral fracture will continue to increase in the near future.

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P342

Short-term smoking cessation improved bone formation in healthy male smokers

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Background and Aim
Smoking increases fracture risk and contributes to COPD-associated osteoporosis. However, its impact on bone metabolism is largely unknown. We thus aimed to determine the effect of smoking cessation on bone metabolism.
Subject and Method
In this prospective study, we recruited 29 healthy Japanese male subjects with smoking habit (37.7 ± 8.2 years old, 16.9 ± 11.3 pack years). Pulmonary function test was done before smoking cessation, and various bone and calcium metabolic markers, inflammatory markers and plasma cotinine levels were examined at 0, 1 and 4 weeks.
Result
Mean FEV1.0/FVC, FEV 1.0% predicted was 81.9% and 101.7%, respectively. Cotinine concentration was 149 ± 119 pg/ml. We confirmed that cotinine levels clearly decreased after cessation except for one subject who failed, and the remaining 28 subjects were analyzed. Smoking cessation caused increases in P1NP at 1 and 4 weeks ($+8.7 \pm 16.7\%$, $P=0.014$; $+10.5 \pm 20.0\%$, $P=0.062$), and under-carboxylated osteocalcin (ucOC) at 1 and 4 weeks ($21.4 \pm 37.9\%$, $P=0.020$; $21.9 \pm 25.6\%$, $P=0.002$). Interestingly, %change of P1NP correlated with %change of intact PTH ($r=0.421$, $P=0.026$ at 1 week: $r=0.556$, $P=0.002$ at 4 weeks). At baseline PTH showed no correlation with 25-hydroxyvitamin D (25D). When the subjects were divided into two groups by 25D levels, the group with lower 25D levels showed larger increases in PTH (19.8% vs. -4.6%) and P1NP (14.2% vs 5.7%) at 1 week. Moreover, negative correlation between PTH and 25D was partially restored after smoking cessation. IL-6, TNF-α and hsCRP were closely interrelated, but consistent changes could not be observed. TRACP-5b levels tended to increase at 4 weeks without significance.
Conclusion
The results indicate that bone formation is suppressed by smoking at least partially in a reversible manner and is recovered after cessation. The mechanism remains to be determined, but appears to be in part associated with impaired PTH secretion due to a disturbed PTH-vitamin D axis.

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P343

Ultrastructure of biomineral of the ramus of mandible in rats after implantation of manganese enhanced hydroxyapatite implants into tibia

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Objectives
The study is aimed at analysis of ultrastructure of biomineral of the ramus of mandible after implantation of manganese enhanced hydroxylapatite into the tibia.
Methods
The study involved 252 male rats. The 1st group comprised intact animals, the 2nd group – animals with 2.2 mm defect in the tibia, and the groups 3 through 6 – the animals with the defects filled with hydroxylapatite enhanced with 0.0, 0.1, 0.25, and 0.5% share of manganese. Biomineral of the ramus of mandible were prepared for X-ray scattering analysis.
Results
Fracture modeling (2.2 mm defect in tibia shaft) leads to instability of ultrastructure of biomineral of the ramus of mandible (enlargement of elementary cells and crystallites, and microtexture coefficient decrease) up to the 90 day of observation. Implantation of pure hydroxylapatite into defect also affects stability of crystal lattice of biomineral of the ramus of mandible; alterations are observed from the 15th to the 30th days of observation and after that crystal lattice began to restore. Manganese enhanced implants significantly reduce negative effects of fracture on instability of ultrastructure of biomineral of the ramus of mandible; the most effective were the implants with 0.25% of manganese. With manganese concentration increase up to 0.25% microtexture coefficient from the 15th to the 60th day was higher than those of the 3rd group by 5.62, 4.19 and 3.70%, and sizes of crystallytes from the 30th to the 180th day were lower than those of the 3rd group by 4.95, 6.14, 6.26 and 3.75% respectively (from here and on, all numeric values are significant with $P<0.05$). Implants with 0.5% of manganese cause manganese intoxication observed as decrease of microtexture coefficient from the 60th to the 180th days of observation with intensity peak at the 90th day of observation (microtexture coefficient decreased from the 60th to the 180th day as compared to the control values of the 3rd group by 3.30, 4.86 and 3.74% respectively).

Conclusions
Application of manganese enhanced implants significantly reduces negative effects of ultrastructure of the biomineral of the ramus of mandible. Implants with 0.25% share of manganese proved to be the most effective while implants with 0.5% share of manganese produced signs of manganese intoxication.

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P344

Ultrastructure of the hipbone biomineral in white rats with defect of the tibia after 60-day administration of sodium benzoate

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Objectives
This study thus was aimed at investigating of ultrastructure of the hipbone biomineral in rats with defect in tibia after 60-day *per os* administration of sodium benzoate (SB) in various concentrations and mexidol (M).
Methods
The experiment involved 280 male thoroughbred rats with initial body weight of 200–210 g. The 1st group (K) comprised animals that received daily *per os* 1 ml of 0.9% solution of NaCl, the 2nd and the 3rd groups (SB1 and SB2) received *per os* 1 ml of SB in dosage of 500 or 1000 mg/kg of body weight, the 4th group (D) comprised animals with defect in both tibiae made when in groups 2 and 3 SB was discontinued. The 5th and the 6th groups (DSB1 and DSB2) comprised the rats who received SB and had defects in tibiae also made after SB discontinue and the 7th and 8th groups also received M in dosage of 50 mg/kg (DSB1M and DSB2M). Observation terms constituted 3, 10, 15, 24, and 45 days after discontinue of experimental influences. For testing of bone biomineral purposes we used X-ray scatter analysis.
Results
Fracture modeling (2.2 mm defect in tibia shaft) on the background intake of SB comparing with group without intake of SB accompanied by enlargement of elementary cells from the 3rd to the 15th day and microtexture coefficient decrease on the 3rd day. It follows that the longest violation occurs of elementary cells forming of hipbone biomineral. In dosage of SB of 500 mg/kg these deviations were respectively 6.68 and 0.14–0.21%, and in dosage of SB 1000 mg/kg – 7.45% and 0.16–0.22%. Defect modeling on the background intake of SB and M in dosage reduces adverse effects of SB on the hipbone biomineral ultrastructure by only 3rd day of observation. With SB dosage 500 mg/kg these accompanied by reducing the size of elementary cells comparing with group without intake of M on 0.15%, and with SB dosage 1000 mg/kg – reducing the size of elementary cells on 0.11% and microtexture coefficient decrease on 3.87% ($P < 0.05$ in all cases).
Conclusions
Defect in tibia after 60-day administration of SB is accompanied by destabilization of ultrastructure of bone biomineral compared with the group without SB. Under dosage of SB of 1000 mg/kg, the severity of the changes was larger than that at a dose of 500 mg/kg. The simultaneous administration of SB and M at dosage of 50 mg/kg smoothed destabilization of ultrastructure of bone biomineral after application defect of the tibia.

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P345

The effects of 60-day sodium glutamate intake and exposure to ionizing radiation on ultrastructure of biomineral of the ramus of mandible in rats

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Objectives
The study is aimed at investigation of ultrastructure of biomineral of the ramius of mandible (BRM) in rats after application of sodium glutamate (SG) and exposure to ionizing 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 eight groups as follows: intact animals for the controls, animals that received *per os* SG in dosage of 30 mg/kg daily for 60 days, animals exposed to IR (total four Grey in four sessions), received Sp in dosage of 250 mg/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 by means of anaesthetized decapitation. For testing of BRM, we used X-ray scatter analysis.
Results
Sixty-day SG intake in dosage of 30 mg/kg results in instability of crystal lattice of BRM yet lattice gradually restores after the 15th day of observation. By the first day after SG discontinue, microtexture coefficient decreased in comparison with the control group by 4.40%. Sixty-day exposure to IR also results in instability of crystal lattice of BRM, which persists up to the 30th day of observation though restoration is insignificant. By the first day after IR discontinue, microtexture coefficient decreased in comparison with the control group by 4.68%. Combined action of SG and IR affects crystal lattice of BRM to a greater extent than those two taken apart and crystal lattice fails to restore in this case. By the first day after SG and IR discontinue, microtexture coefficient decreased in comparison with the group with only SG intake by 4.86%. Application of Sp in dosage of 250 mg/kg reduces negative effects of SG and IR on crystal lattice of BRM. Restoration of crystal lattice of BRM (i.e. order degree increase and widening of exchange area) was also faster yet correction efficiency for combined influences was lower than for separate influences.
Conclusions
Sixty-day application of SG in dosage of 30 mg/kg and exposure to IR (total four Grey in four sessions) and their combined action results in instability of crystal lattice of BRM that expands even to readaptation period. This fact urges searching for medication and prophylactic measures for such a state. According to our findings Sp in dosage of 250 mg/kg well satisfies this demand.

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P346

Macroelemental contents of skeletal bones after 60-day exposure to toluene vapors in rats of different ages

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Objectives
This experimental study is aimed at investigating of macroelemental contents of skeletal bones in rats after 2-month inhalation of toluene vapors (Tol) and administration of thiotriazoline (Th) and *Echinaceae tinctura* (*ET*) as medication.
Methods
The experiment involved 420 male rats (young, mature and old) each separated into: intact animals, animals that received daily Tol inhalations as a single 5-h exposure to 10 MPC for 60 days and the groups 3 and 4 received inhalations of Tol and intraperitoneal Th in dosage 117.4 mg/kg and *per os ET* in dosage of 0.1 mg of active component per 100 g of body weight. Upon expiration of observation terms tibia, hipbone and third lumbar vertebra were prepared for chemical analysis.
Results
Exposure to Tol result in hyperhydratation, demineralization and macroelements composition destabilization in tibia, hipbone and third lumbar vertebra. Upon cessation of Tol exposure, shares of mineral content, Ca and Ca/P ratio were lower than those of the control group by 6.13–7.78, 11.77–12.86 and 16.96–18.40%, in adult animals – by 5.43–6.65, 9.75–10.97 and 13.46–16.20% and in old – by 5.62–6.65, 8.45–8.94 and 11.86–12.55% ($P < 0.05$ in all cases). In readaptation period, macroelement levels of tibia, hipbone and vertebra in young animals restored after the 15th day, in adult rats alterations persisted up to the 30th day and gradually reduced yet by the 60th day alterations were still observed. In old animals alteration persisted throughout of the whole observation period.
After administration of Th during Tol inhalations in comparison with non-medicated animals macroelement contents of tibia, hipbone and third lumbar vertebra in young animals restored after the 15th day of observation, in adult animals restoration signs were registered in the period from the 15th to the 60th days of observation and in old animals – from the 7th to the 60th days of observation. After administration of *ET* changes of macroelement contents of tibia, hipbone and vertebra in young animals as compared to the controls restored after the 15th day of observation, in adult and senile animals changes were registered by the 30th and the 60th days of observation.
Conclusions
Sixty-day inhalation of Tol results in macroelements composition destabilization of bones. Application of Th or *ET* reduces negative effects of Tol. We proved Th to be more effective than *ET*.

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P347

Development of Korean Fracture Risk Score predicting osteoporotic fracture risk: analysis of data from the Korean National Health Insurance Service

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Background
Asian-specific prediction model for estimating the individual risk of osteoporotic fracture was rare. We aimed to develop a Korean fracture risk prediction model using clinical risk factors and assess external validity of the final model.
Methods
A total of 718,306 Korean men and women aged 50–90 were followed for 7 years in national system based cohort study. Fifty percent were randomly assigned to the development dataset and 50% to the validation dataset. Clinical risk factors for osteoporotic fracture were assessed at biennial health check. Data on osteoporotic fracture in follow-up period were identified by using the ICD-10 codes and the nationwide database of the National Health Insurance Service (NHIS).
Results
During the follow-up period, 19,840 osteoporotic fractures were reported (4889 in men and 14,951 in women) in the development dataset. The assessment tool, called the Korean Fracture Risk Score (KFRS) is comprised of a set of nine variables that include age, BMI, recent fragility fracture, current smoking, high alcohol intake, lack of regular exercise, recent use of oral glucocorticoid, rheumatoid arthritis and other causes of secondary osteoporosis. The KFRS was shown to be predictive of osteoporotic fracture over 7 years. This score was validated using independent dataset. When we compared the mean predicted scores applying the KFRS with the observed risks at 7 years within each 10th of predicted risk, there was close correspondence for overall fracture.
Conclusion
We developed a Korean specific prediction model for osteoporotic fracture. The KFRS can predict risk of fracture in primary population without BMD testing and therefore suitable for use in both clinical setting and for self-assessment. A web site is available at http://www.nhis.or.kr.

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P348

The relationship between osteoporosis and urine ACR in postmenopausal women with type 2 diabetes

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Purpose
Osteoporosis is a progressive bone disease that is characterized by a decrease in bone mass, density and destruction of microstructure, which can lead to an increased risk of fracture. Although many studies have been published about relationship between end-stage renal disease and osteoporosis, but research on the relationship between proteinuria and the prevalence of osteoporosis is still lacking.
Methods
We assessed 91 postmenopausal women with type 2 diabetes who visited our hospital from January 2009 to January 2012.
Results
Among 91 patients, prevalence of osteoporosis and osteopenia was 35.2% (32 cases) and 33% (30 cases) according bone mineral density. The patients with microalbuminuria and macroalbuminuria (ACR $\geq$ 30) have a significantly higher incidence of osteoporosis compared to subject with normoalbuminuria ($P < 0.05$).
Conclusion
This study indicates that urine ACR may be useful biomarkers for increased risk of osteoporosis in postmenopausal women with type 2 diabetes who has been linked to higher urine ACR levels.

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Relationships between the trabecular bone score (TBS) and glucose metabolism indices in healthy postmenopausal women

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Purposes
The trabecular bone score (TBS) is an indicator of cancellous bone microstructure obtained from image analysis with lumbar spine dual-energy X-ray absorptiometry (DXA) scans. A decrease in TBS is considered to be a risk factor for vertebral fracture that is independent of BMD. At the same time, it has been reported that, BMD was higher and TBS was lower in women with type 2 diabetes mellitus (DM), and a decreasing TBS is risk factor for fracture. However, the relationships between TBS and glucose metabolism indices in healthy people are unclear, and this study was conducted to elucidate these relationships.
Method
The subjects were 214 healthy postmenopausal women with HbA1c $\leq$6.2% who underwent an osteoporosis examination. On blood tests, fasting plasma glucose (FPG), HbA1c, Ca, P, Cr, PTH, 25-hydroxy vitamin D [25(OH)D], P1NP, and CTX were measured. Lumbar (L2-4) BMD and femoral neck (FN) BMD were measured with DXA, and TBS (L1-4) was calculated.
Results
The subjects' mean values were age 63.2 $\pm$ 7.5 years, BMI 22.7 $\pm$ 3.0 kg/m^2, FPG 90 $\pm$ 8 mg/dl, HbA1c 5.6 $\pm$ 0.3%, PTH 45.5 $\pm$ 14.5 pg/ml, 25(OH)D 16.3 $\pm$ 4.3 ng/ml, BMD (L2-4) 0.846 $\pm$ 0.146 g/cm^2 (*Z* score 0.3 $\pm$ 1.0), and FN 0.620 $\pm$ 0.093 g/cm^2 (*Z* score 0.1 $\pm$ 1.0). TBS was 1.317 $\pm$ 0.073. An investigation of the correlation between TBS and each of these factors revealed that TBS was negatively correlated with age, PTH, and HbA1c, and significantly positively correlated with BMD, and 25(OH)D. On multiple regression analysis, HbA1c still showed a significant negative correlation with TBS even after adjusting for age and BMI ($r = -0.155$, $P < 0.05$).
Conclusion
These results suggest that very mild glucose intolerance, even within the normal range, may be associated with deteriorating cancellous bone microstructure in postmenopausal women.

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Bone mineral density in women with Parkinson's disease

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Aim
The aim of the research is to define the bone mineral density in patients, with Parkinson's disease.
Methods
We examined 12 women with Parkinson's disease and 12 healthy women of appropriate age (average age: -63.6 ± 6.25 vs 62.2 ± 6.83 years, $P = 0.5$). The duration of Parkinson's disease was at list 5 years. All patients reseived levodopa.
Results
BMD of women with Parkinson's disease was significantly lower compared with BMD of women of control group on the level of total body (*T*-score $= -1.86 \pm 1.32$ vs -0.71 ± 1.48, $P < 0.05$, *Z*-score $= -0.35 \pm 0.93$ vs 0.51 ± 1.05, $P < 0.05$), lumbar spine (*T*-score $= -1.56 \pm 1.22$ vs 0.10 ± 1.63, $P < 0.05$, *Z*-score $= -0.66 \pm 0.87$ vs 0.72 ± 1.53, $P < 0.05$) and at the distal forearm (*T*-score $= -1.87 \pm 1.32$ vs 0.71 ± 1.47, $P < 0.05$, *Z*-score $= -0.51 \pm 1.05$ vs 0.38 ± 1.22, $P < 0.05$). Hip BMD was not different from control group. It is important to note that one woman with Parkinson's disease has two hip endoprostheses after femoral neck fractures.
Conclusion
BMD in women with Parkinson's disease is significantly lower than in healthy wowen of the same age.

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Gender differences in presentation and outcomes in older hip fracture patients

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Introduction
Hip fracture is a significant health problem with 25–30% occurring in men. Outcome differences between the genders have been documented, particularly mortality rates.
Aim
To prospectively investigate outcomes of elderly hip fracture patients regarding mortality, recovery of function, quality of life, incidence of osteoporosis, osteoporosis knowledge, medication adherence, nutritional status and fear of falling.
Methodology
A longitudinal study of hip fracture patients attending the study site between June 2008 and 2010. Participants were contacted 4 monthly for 15 months. Gender comparisons were made. Data analysed with SPSS.
Results
$n=226$.71%:29% female:male. Mean age 81 years female, 76 years male. Men higher ADL score pre-fracture ($P=0.059$), no difference at 15 months. Men more mobile at 11 and 15 months ($P=0.003$, $P=0.006$ respectively). Women had greater FOF ($P=0.005$) and increased risk of malnutrition at 3 months ($P=0.007$), no difference at 15 months. No significant difference noted in hospital stay, discharge destination, pain, QOL, fracture type, recovery in basic and instrumental ADLs or residency at 15 months. More women had low serum calcium ($P=0.02$) and vitamin D deficiency ($P=0.005$). Mortality significantly higher in men at 6 months ($P=0.03$).
Conclusion
Hip fracture is a problem affecting both genders. As with other studies, men were younger, less likely to live alone and significantly more likely to die within first 6 months. The increased recovery in mobility for men identified may be due the fittest men surviving to functional assessments at 15 months.

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Atypical femur fractures (AFF): a case-control study

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Atypical femur fractures (AFF) have been associated with antiresorptive therapy. In a retrospective case-control study, we identified AFF using ASBMR 2013 criteria. Femoral shaft fractures were identified using ICD9 codes. We screened 1479 radiographs. Radiographs were excluded for high-energy trauma, tumor, or periprosthetic fracture. Two radiologists blinded to treatment scored 482 radiographs for AFF features, and jointly adjudicated discrepancies. The required AFF feature, low-energy trauma fractures between the lesser trochanter and supracondylar flare, occurred in 98 patients. These 98 had additional major AFF feature prevalence of: 64% lateral or uncertain origin; 98% complete/incomplete lateral fracture; 71% minimally comminuted/noncomminuted; 49% periosteal/endosteal reaction. A total of 57 (58%) of the femoral shaft fractures met AFF criteria (16% of the 98 having four major features, and 42% having five major features). Initial radiologist score agreement for AFF was 93%. All 11 patients with bilateral femoral shaft fractures had AFF (ten with bilateral AFF).
Fifty-four AFF cases were race- and sex-matched to 108 controls having non-AFF femoral fractures. Clinical variables were similar between controls having non-AFF fractures in the femoral shaft ($n=29$) and those with non-shaft control fractures ($n=79$). Drug use was determined primarily from dispensing records and augmented by chart review. AFF were associated with bisphosphonates within 5 years, glucocorticoid use at fracture occurrence, prodromal pain and lower alkaline phosphatase. Age, cortical thickness, prior osteoporotic fracture, other medical conditions and proton pump inhibitors were not associated with AFF.
Over half of the low-energy trauma femoral shaft fractures met the ASBMR 2013 AFF criteria. Though strongly associated with bisphosphonates, additional risk factors should be considered for AFF.

	Cases ($N=54$)	Controls ($N=108$)	OR (CI)	P-value
Age (s.d.) years	72.8 (11.0)	74.0 (16.5)	1.00 (0.97–1.02)	0.67
Prodromal pain	39%	5%	13.04 (3.87–43.88)	<0.0001
Bisphosphonates in 5 years	57%	8%	13.69 (4.81–38.99)	<0.0001
Oral glucocorticoids	17%	3%	6.00 (1.62–22.16)	<0.01

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Dietary potassium intake is beneficial to bone health in Korean adults with low dietary calcium intake: the Korean National Health and Nutrition Examination Survey (KNHANES) (2008–2011)

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Nutrition is a major modifiable factor that affects bone health. Dietary potassium may act as an alkaline source by neutralizing the acid load and reducing calcium loss from bone.
We aimed to evaluate the association between dietary potassium intake and bone mineral density (BMD) in the Korean population. We analyzed data from the Korean National Health and Nutrition Examination Survey (KNHANES) A total of 3135 men aged >50 years and 4052 postmenopausal women were included. Lumbar spine, total hip, and femur neck BMD were measured using dual energy X-ray absorptiometry. The daily food intake was assessed using a 24-h dietary recall.
When we divide the subjects into tertiles based on the intake of potassium intake, the higher potassium intake group in men had significantly higher BMD at the total hip (0.891 ± 0.013, 0.907 ± 0.013, 0.908 ± 0.014 g/cm^2, $P=0.004$) and femur neck (0.720 ± 0.013, 0.735 ± 0.013, 0.741 ± 0.013 g/cm^2, $P=0.001$), as compared to the other groups. Postmenopausal women in the higher potassium intake tertile group had significantly higher femur neck BMD as compared to those in the lower tertile groups (0.615 ± 0.003, 0.619 ± 0.002, 0.627 ± 0.003 g/cm^2, $P=0.034$). In subgroup analysis according to dietary calcium intake, an association between dietary potassium intake and BMD was observed only in cases with low dietary calcium intake (<800 mg/day).
Dietary potassium intake was positively associated with BMD in men aged >50 years and postmenopausal women, indicating the beneficial effects of dietary potassium intake on bone health, particularly among those with low dietary calcium intake.

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Bone metabolism in patients with anorexia nervosa and amenorrhea

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Context
Anorexia nervosa (AN) occurs predominantly in females, most frequently during adolescence and it is generally associated with amenorrhoea. AN increases the risk for impaired bone health and for low bone mineral density (BMD) by a mechanism which has not been yet well defined.
Objective
The study purpose was to further characterize bone metabolism in AN with amenorrhoea, and so with oestrogen deficiency
Design
AN patients were compared with healthy females matched for age and with postmenopausal women as a model for oestrogen deficiency.
Study popultion
The study population included 81 females with AN: 48 young adults and 33 adolescents. All had amenorrhoea for at least 6 months and none of them had serum 25 OH vitamin D lower than 20 ng/ml. The control groups included age matched healthy young ladies ($n=20$), 17 healthy adolescents and 21 postmenopausal women.

Methods
We studied bone turnover markers, intact parathyroid hormone, 25 hydroxy-vitamin D, Sclerostin (SOST) and Dickkopf-related protein 1.
Results
AN participants had higher C-terminal telopeptide of type I collagen (CTX) levels than both control groups. AN adolescents had CTX higher than AN young adults. In postmenopausal women intact N-propeptide of type I collagen was higher as compared with in each other group.
In AN groups Dickkopf-related protein 1 was significantly lower than the two control groups. No differences were found in sclerostin except in adolescents. In AN adolescents DXA BMD at femoral sites were higher than in AN young adults and it was found positively correlated with body weight ($P<0,01$) and with fat mass evaluated by DXA ($P<0,01$).
Conclusion
Our data suggest that in AN women with amenorrhoea bone resorption is as elevated as in postmenopausal women but bone formation is relatively depressed. The consequent remodelling uncoupling is considerably more severe than that occurring after menopause.

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The correlation between number and population of bone marrow endothelial progenitor cells with bone mass and bone metabolism in the elderly

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Objective
Endothelial progenitor cells (EPCs) have the potential ability to differentiate into vascular endothelial cells and osteoblasts for angiogenesis and osteogenesis, however, the correlation between number and population of bone marrow EPCs with bone mass and bone metabolism in elderly is unknown.
Methods
Trabecular bone were extracted from 11 patients with fragility fracture and eight patients with osteoarthritis during artificial hip replacement surgery, and EPCs separated from bone marrow were prepared for flow cytometry analysis. All the patients took DEXA scan, and bone metabolism detection. The impact of clinical data such as age, BMI, bone mass and bone metabolism markers on the number and population of bone marrow EPCs in the elderly were analyzed.
Results
There was no significant difference of age and BMI between fragility fracture patients and osteoarthritis patients. The total number of bone marrow EPCs and number of mature EPCs in fragility fracture patients were significantly less than that in osteoarthritis patients (0.48 ± 0.35 vs 1.80 ± 1.01, $P=0.001$; 52.28 ± 21.20 vs 77.13 ± 19.15, $P=0.042$), and the bone mass of femur neck and total hip (0.54 ± 0.14 vs 0.76 ± 0.21, $P=0.021$; 0.65 ± 0.14 vs 0.84 ± 0.15, $P=0.026$) as well as serum 25(OH)D level (4.50 ± 1.56 vs 23.80 ± 2.88, $P=0.033$) in fragility fracture patients were significantly lower than those in osteoarthritis patients, however serum PTH lever (73.60 ± 1.84 vs 32.20 ± 0.98, $P=0.035$) was significantly higher in fragility fracture patients than that in osteoarthritis patients. There are significantly negative correlation between age with number of mature EPCs ($r=-0.594$, $P=0.015$), and positive correlation between bone mass in femur neck and total hip with number of mature EPCs ($r=0.847$, $P=0.008$; $r=0.925$, $P=0.034$), and negative correlation between bone mass in total hip with number of premature EPCs ($r=-0.817$, $P=0.047$). However, BMI, 25(OH)D and PTH did not show any correlation with number of bone marrow EPCs.
Conclusion
Bone marrow EPCs could influence bone mass via regulating bone metabolism directly or indirectly in the elderly.

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The added value to FRAX of gait speed and tests of postural balance

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Objectives
Postural balance and gait speed are risk factors for falls and hip fractures independent of bone mineral density (BMD). The standard risk assessment tool, FRAX, does however not include any estimate of fall risk. Identifying high-risk individuals correctly is be of great clinical importance why further improvement of FRAX would be clinically valuable. The primary aim of this study was to see if one-leg standing time, the maximum number of steps on a line or gait speed could improve the predictive ability of FRAX for hip fractures.
Material and methods
One-leg standing time with eyes open, the number of consecutive steps on a straight line without stepping beside and gait speed over $15+15$ m the line, was measured at baseline in 351 women aged between 69 and 79 years. Fracture data for the following 10 years was obtained from health care registers.
Results
Gait speed was the most valuable addition to FRAX. If 5% hip fracture risk was used as cut-off, categorical NRI was 0.24. The area under curve (AUC) for the receiver operating characteristic (ROC) increased from 0.59 to 0.71 for hip fractures when gait speed was added to FRAX. The risk of a hip fracture was increased 8.3 if gait speed was <0.8 m/s compared to $\geqq 0.8$ m/s (HR 8.3, 95% CI 3.62–18.98). This HR was independent of FRAX-risk.
Conclusion
Gait speed could be a valuable addition to FRAX and it seems to be more valuable than tests of postural balance in that aspect.

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Older urban Black South African women are increasingly at risk of low bone mass and high bone turnover

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South African studies indicated that urban Black and White women have similar bone turnover. However, urban Black women had low bone mass and greater exposure to lifestyle risk factors for bone disease. The purpose of this prospective study was to assess changes in bone turnover, parathyroid hormone (PTH) levels, bone health (forearm, hip and lumbar spine bone mineral density) in urban black South African women over 3 years. Black, urban postmenopausal women ($n=144$, >50 years) from the North-West Province, South Africa were recruited. Forearm bone density measurements (BMDDTX) were performed at the distal and ultra-distal sites in the non-dominant arm (DTX-200Osteometer MediTech). Conventional central bone density (BMDDXA) scans of the lumbar spine (L1-L4) and hip were performed using a Hologic Discovery-W. Blood concentrations of C-Telopeptide of Type I collagen (CTx), PTH and 25 (OH) D3 were assessed (Roche Elecsys) and physical activity (PA) was assessed using a validated questionnaire. Over the three years CTx and PTH levels increased ($P<0.001$), and 25 (OH) D3 levels reduced ($P<0.001$), while hip bone density decreased significantly ($P<0.001$) and had a medium effect ($r=0.38$). In multiple regression the predictors of % change in PTH were CTx and PTH at baseline (negative association), and height, PA score and C-reactive protein (CRP) at baseline (positive association). CTx and magnesium intake at baseline (negative associations) predicted % CTx change. Age and PA score (negative) and CRP (positive association) were significant predictors of % change in left hip BMD. Age and PA score were also negatively associated with % change in forearm BMD. Higher CRP at baseline was associated with greater % changes in PTH and hip BMD. In general, inflammation contributed to greater decreases in BMD and increased bone turnover, whereas a higher physical activity score in 2010 was associated with smaller decreases in BMD among these women.

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Treatment pattern and its cost of vertebral fracture among elderly patients in Korea

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Objectives
Vertebral fracture (VF) is a hallmark of osteoporosis, and it increases morbidity, mortality and large amount of medical cost. In this study, we aimed to describe the treatment pattern and estimate the cost of VF among elderly patients in Korea.

Methods
Using Korean national healthcare claims database, we analyzed a 2% sample of patients aged ≧ 50 years between years of 2008 and 2012. New VF was identified on the basis of selected ICD-10 codes in patients who did not have VF in past 1 year, and medical cost includes in-hospital and outpatient medication cost. After estimating incidence of VF, treatment pattern and medical cost in first and second year in VF were compared according to age and gender.
Results
The incidence of VF was much higher in female (616 and 1597 per 100 000 in male and female, respectively). The 14% of patients underwent vertebroplasty or kyphoplasty, 42% of patients needed hospitalization for pain control, the other 43% were treated without hospitalization. Patients aged 50–59 years showed lowest proportion for vertebroplasty or kyphoplasty (1.8% in male and 8.2% in female), the proportion was increased with the age (7.9% in male and 12.2% in female aged 60–69 years; 16.7% and 17.5% in 70–79 years; 20.0% and 17.7% ≧80 years). Patients underwent vertebroplasty or kyphoplasty paid the largest medical cost in first year (€1532 in male and €1514 in female), followed by hospitalization (€762 and €687) and without hospitalization (€305 and €262). In addition, medical cost according to age group did not show significant trend, whereas proportion of medical cost in second year was increased with age.
Conclusion
In Korean patients with VF, 14% of patients underwent vertebroplasty or kyphoplasty, and the proportion was increased with age. In addition, older patients tend to pay more medical cost for longer period.

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Glucocorticoid acts differently on vertebral and hip fractures in Korean RA patients using National healthcare claims database

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Background and objectives
Fracture in rheumatoid arthritis (RA) patient is more frequent than general population. One of the important reasons is use of glucocorticoid (GC) for treatment of RA. In this study, we aimed to identify the effect of GC on fracture of different site.
Methods
Among RA patients ≧19 years, we established a retrospective cohort using Korean national healthcare claims database from Jan 2010 to Dec 2010, and then followed up until Dec 2013. RA patients who experienced fracture in year 2009 were excluded. Fractures were identified on the basis of selected ICD-10 codes and information about clinic visit until last visit. Information about oral GC use was collected until incidence of fracture or last visit. In multivariable logistic regression analysis, each of variables such as duration, mean dose, and highest daily dose of GC were adjusted for age, gender, payer type, type of institution, physician's specialities, comorbidities and medication.
Results
The 11 599 fractures in 9964 RA patients were observed among total of 138 240 RA patients. The 68% of patients used oral GC more than 3 months. Mean dose of GC was 6.1±4.7 mg and their highest daily dose was 16.20±15.8 mg. In adjusted analysis, duration of GC ≧6 months (OR 1.36, $P<0.01$ for total fracture; OR 1.76, $P<0.01$ for vertebral fracture), mean dose of GC ≧2.5 mg (OR 1.17–1.34, $P<0.01$ in total fracture; OR 1.37–1.71, $P<0.01$ in vertebral fracture) and highest daily dose of GC ≧10 mg (OR 1.22–1.33, $P<0.01$ in total fracture; OR 1.23–1.75, $P<0.03$ in vertebral fracture) increased the risk of total and vertebral fracture. However, in hip fracture, neither duration nor dose of oral GC increased the risk.
Conclusion
Use of oral GC increased the risk of total and vertebral fracture, however, it did not increase the risk of hip fracture in RA patients.

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Warfarin use and changes in bone mineral density in the population-based canadian multicentre osteoporosis study (CaMos)

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Background
Osteocalcin has an important role in bone metabolism. Uncarboxylated osteocalcin predicts risk for hip fracture and lower bone mineral density (BMD). Warfarin inhibits carboxylation of osteocalcin, providing a mechanistic link between warfarin and impaired bone metabolism. Studies examining the relationship between warfarin use and BMD have been inconsistent. The goal of this study was to further characterize this relationship.
Population
CaMos is a population-based, prospective cohort of the Canadian population followed since recruitment in 1995–1997. Participants ($n=4740$ female, $n=1905$ male) underwent BMD testing at L-spine (L1-4), total hip (TH) and femoral neck (FN) and an interviewer-administered questionnaire at years 0, 5 and 10.
Methodology
Cross-sectional analysis of year 0 data examined warfarin users ($n=128$) and non-users ($n=6517$). Longitudinal analysis examined the continuous users of warfarin at year 0 and 5. A multivariate linear regression model was used to analyze mean change in BMD in continuous warfarin users vs non users at year 0 to 5, year 5 to 10 and year 0 to 10.
Results
At year 0 there was no significant adjusted difference in BMD among warfarin users (vs non-users) at TH ($P=0.064$), FN ($P=0.755$), or L1-4 ($P=0.156$). Multivariate analysis of continuous warfarin users showed a statistically significant greater decrease in BMD in continuous warfarin users (vs non-users) at years 5 to 10 at TH (-0.013 g/cm^2; $P=0.029$) and FN (-0.012 g/cm^2; $P=0.035$) and at year 0 to 10 at TH (-0.024 g/cm^2; $P=0.017$) and FN (-0.024 g/cm^2; $P=0.008$).
Conclusion
This study demonstrates an association between warfarin use and reduced BMD in continuous warfarin users over a 10-year period. Ongoing longitudinal studies are needed to further clarify the issue of warfarin use and bone metabolism, with focus on fracture rates, and whether long-term warfarin users would benefit from lifestyle or pharmacological interventions for bone protection.

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The relationship of serum serotonin levels to the rate of bone loss and fractures in men

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Recent genetic studies in rodents have revealed that circulating serotonin plays a key role in regulating bone formation and skeletal mass. However, the reported effects of circulating serotonin on bone mass in humans have been conflicting. We determined whether circulating serotonin levels influenced the rate of bone loss and fractures in males.
We assessed the effect of serum serotonin on bone loss rate in a population-based cohort of 202 ambulatory males aged 56–70 years who were followed up for a median duration of 3.7 years. Serum serotonin levels were assayed, and the Timed Up-and-Go test (TUGT) performed, at baseline. Dual energy X-ray absorptiometry was performed both at baseline and during follow up. Fracture prevalence was assessed using questionnaires.
The serotonin levels were inversely associated with the lumbar spine bone mineral density (BMD) ($r=-0.174$, $P=0.028$) at baseline. No association was evident between the BMDs of the femoral neck or total hip, and serotonin level. The annual rate of bone loss from the lumbar spine, the femoral neck, and the total hip were 0.01, 0.46, and 0.46%, respectively. The baseline serum serotonin level did not predict the bone loss rate at any skeletal site. Lower limb disability evident

upon TUGT at baseline predicted bone loss from the total hip. No significant difference of serotonin level was observed between subjects with and without fractures. The serum serotonin level was not associated with the rate of bone loss in elderly males. Thus, the circulating serotonin level does not reliably predict bone loss.

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Differential influence of social network upon osteoporosis affected by intimacy in Korean elderly women

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The present study figured out the relationship between social network and osteoporosis among elderly women. Social network could be beneficial to one's health since it works as social capital providing several supports. On the other hand, social network can also be a source of social burden aggravating one's health. To examine the seemingly contradicting associations, Korean Urban Rural Elderly Study (KURE) data was analyzed. There were 1938 older women aged over 65, of whom 882 (45.5%) were diagnosed to have osteoporosis. The results showed that there is a U-shaped relationship between network size, the number of people in one's social network, and osteoporosis ($P=0.03$). Specifically, predicted probability of having osteoporosis decreases from 48 to 37% until network size 4. Beyond the ideal threshold, however, the predicted probability increased to 57% when subjects have more than six people in their social networks. Negative part of U-shaped influence was explained by poor intimacy. For those with friendly social network, increasing network size linearly reduced predicted probability of having osteoporosis from 47 to 30% ($P=0.048$). For those with unfriendly social network, on the other hand, curvilinear relationship was discovered ($P=0.004$). The probability decreased from 49 to 39% until network size 4, after which it dramatically increased to 74%. The results imply that maintaining favorable social network is beneficial for the elderly women to manage their bone mass, while too large network full of uncomfortable relationships could be even harmful.

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Lower FRAX scores but similar femoral neck aBMD in UK dwelling postmenopausal South Asian women as compared with same age Caucasian women

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It is unclear as to whether western dwelling South Asian (SA) postmenopausal women have a different fracture risk to that of the native Caucasian (C) population. Moreover, the WHO Fracture Risk Assessment Tool (FRAX) has not been used previously to compare predicted risk of fractures in western dwelling South Asian women with same-age Caucasian women. This analysis used data from $n=35$ SA [mean (S.D.) age = 59 (6) years] and $n=136$ C [mean (S.D.) age = 61 (5) years] postmenopausal women in the D-FINES I study (UK, 2006–2007). FRAX score was calculated using femoral neck arial BMD (FN aBMD) and background demographic data, via the online tools at www.shef.ac.uk/FRAX/. The SA women were scored on both the India and UK tools as it was unclear which was likely to be the best epidemiological fit. There was no difference ($P>0.05$) between the two ethnic groups for FN aBMD ($P=0.44$). However, the 10 year fracture risk (%) was lower in the SA group (using the India tool) than the C group by 50–60% (see table below). Similar results were obtained when using the UK tool in the SA group, with 28–50% lower risk of fractures in SA than in C. The reduced FRAX score in the SA group has not been reported previously and may be due to a variety of lifestyle and medical factors, as well as possible differential reporting of family history of fracture. Further research is warranted with respect to future fracture risk in this ethnic group. These data will be helpful in the clinical setting.

Group (Tool) *n* 10 year probability:	C (UK) $n=136$		SA (India) $n=35$			SA (UK) $n=35$	
	Median	IQR	Median	IQR	*P**	Median	IQR
O. Fracture %	6.5	4.4	2.5	2.5	<0.001	4.7±	2.5
H. Fracture %	0.4	0.8	0.2	0.5	0.006	0.2±	0.6
	Mean	SD	Mean	SD	P≠		
FN aBMD (g/cm^2)	0.76	0.11	0.77	0.1	0.44	–	–

*Mann–Whitney: C (UK) vs SA (India), O. = Osteoporotic, H. = Hip, ≠ ANCOVA *P* value for BMI adjusted data. ± Statistically different from SA (India) and C (UK) using Wilcoxon test ($P<0.005$).

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Peak bone mass and quantitative ultrasound bone properties in young adulthood: a study in the PEAK-25 cohort

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Peak bone mass, typically reached in the third decade, is the highest bone mass acquired after completion of normal growth. Attaining a higher young adult bone mass may contribute to a lower risk of fragility fractures later in life. Few studies have specifically investigated quantitative ultrasound (QUS) in relation to peak bone mass in young adult women. The study objectives were to describe normative QUS values for 25 year old women and how extremes of body weight relate to QUS.

The QUS variables speed of sound (SOS), broadband ultrasound attenuation (BUA) and stiffness index (SI) were measured at the calcaneous in the population based PEAK-25 cohort ($n=1061$; age 25.5±0.2). Written informed consent was obtained from all participants. Based on the QUS manufacturer supplied reference values, young adult % values (YA%) were calculated. Analyses were performed (i) in the whole cohort and (ii) comparing women in the lowest and highest octiles for weight or body mass index (BMI).

Compared to the reference population, young adult SOS values in the PEAK-25 cohort were higher (108±18%). SOS relates to BMD and mirrors the previous finding of comparatively high BMD in the cohort. BUA values, which relate to bone complexity, were lower (90±14%). Body weight or BMI did not correlate with SOS. In the cohort overall correlations between BUA, weight and BMI were Pearson's $r=0.261$; $r=0.197$ respectively; $P<0.001$). In the low-weight group, *r*-coefficients were higher ($r=0.313$; $r=0.268$; $P<0.05$). In contrast, in the high-weight group correlation between BUA, weight and BMI tended to be small, negative and non-significant.

In conclusion, in these 25-year old women, a comparatively high peak bone mass appears to be offset by less complex bone structure. This may have serious implications for osteoporosis assessment and future fracture risk.

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Prevalence and related factors assessment of osteoporotic fracture in rural population: the Korean Genomic Rural Cohort study

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Objective

Due to the increase in the elderly population, osteoporosis and related fractures are increasing and causes serious social problems such as lower quality of life of seniors and economic loss. This study is aimed towards the general population in rural areas for prevalence and related risk factors of osteoporotic fracture.

Materials and methods
The research comes from the Korean Rural Genomic Cohort study consisting of 10 111 people, 4090 men and 6021 women, ranging from 40 to 70 years old from rural areas in Korea. The questionnaire results show that 907 men experienced fractures with 208 fractures due to osteoporosis. 1058 women experienced fractures with 603 fractures due to osteoporosis. Fractures and related clinical factors were collected through questionnaire, bone mineral density was measured with heel quantitative ultrasound, and osteoporotic fracture groups were statistically analyzed.
Results
Osteoporotic fractures prevalence is 8% overall, with women having double the prevalence of women at 10%, men had 5%. In age groups, men 40–49, 50–59, and at least 70 years old had prevalence of 4.5, 5.7, and 7.3% respectively. For women, 40–49, 50–59, and at least 70 years old had prevalence of 4.8, 9.0, and 16.8% respectively. Comparing osteoporotic fracture and non-fracture groups, age and bone density T-scores were statistically significant ($P<0.05$) factors for men while age, T-score, waist–hip-ratio, muscle mass, and time period after menopause were statistically significant factors for women. After adjusting for age, both men' bone density T-score (OR = 0.855, 95% CI: 0.768–0.951) and women' bone density T-score (OR = 0.822, 95% CI: 0.767–0.879) were statistically significant. For women, family history of osteoporotic fracture and smoking habits were correlated with osteoporotic fractures.
Conclusion
Osteoporotic fracture prevalence is 8%, with women having significant correlation factors in age, bone density T-score, family history of osteoporotic fracture, and smoking habits.

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Effect of recent spinal cord injury on the OPG/RANKL system and its relationship with bone loss and antiosteoporotic response to denosumab therapy: preliminary results

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The aims of this study were to analyze the role of the regulators of bone remodeling, OPG and RANKL, in the bone loss associated with recent spinal cord injury (SCI) as well as the effect of antiosteoporotic therapy with denosumab in these bone regulators in a prospective study.
Patients and methods
Twenty-three male patients (aged 18–67 years (mean 36 ± 16 years)) with recent (< 6 months) complete SCI were prospectively included (43.5% paraplegic, 53.5% tetraplegic). Serum levels of OPG and RANKL (Biomedica, Vienna, Austria), bone turnover markers (PINP, bone ALP, sCTX) and BMD were assessed at baseline (99 ± 30 days after SCI), prior to initiating antiosteoporotic treatment (14 ± 4 months post-SCI) and during antiosteoporotic therapy with denosumab (6 months after initiating treatment). The results were compared with a healthy control group.
Results
At baseline, SCI patients showed a significant increase in RANKL serum levels compared to controls (3.4 ± 1.7 vs 2.3 ± 1.6 pg/ml, $P=0.022$) which correlated with days-since-SCI ($r=0.589$, $P=0.005$) and became undetectable after denosumab treatment in 67% of the patients ($P=0.001$). OPG serum levels were similar to controls at baseline (87.7 ± 38.7 vs 71.7 ± 25.4, $P=0.1$) and did not change with denosumab treatment. No differences were observed in RANKL and OPG levels on comparing tetraplegic vs paraplegic patients. Neither were RANKL or OPG levels related to the increase in bone loss and bone turnover markers after SCI. Patients with undetectable RANKL serum levels after denosumab treatment did not present further sublesional bone loss but increased total hip BMD (+2.5 ± 1.3%, $P=0.005$), and bone markers markedly decreased (PINP: −53%, $P=0.007$; sCTX: −68%, $P=0.005$).
Conclusion
This study shows that short-term after SCI there is an increase in RANKL serum levels which become undetectable after denosumab treatment. The preventive effect of denosumab on sublesional bone loss further suggests a contributory role of RANKL in this clinical process.

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FGF23 and SCL are expressed in carotid plaques and the association between their circulating fractions and fractures differs in relation to comorbidity in elderly individuals

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Sclerostin (SCL) and FGF23 are osteocyte-secreted factors with a major role in bone homeostasis. Despite their skeletal effects and their association with fracture risk in some studies, variations in circulating levels were also described in patients with diabetes (DM), chronic renal failure (CRF) and/or cardiovascular disease (CVD). In order to provide further insight on the relationship between these osteocyte-derived factors, osteoporosis and cardio-metabolic disorders we assessed their circulating fractions in 1353 elderly subjects from two cohorts specifically designed to assess the prevalence of cardio-metabolic comorbidities and morphometric vertebral fractures (MVFs). Moreover, serum and tissue samples of 30 patients who underwent carotid endarterectomy were analyzed. In both cohorts, SCL levels were positively correlated with CTX, creatinine and inversely with physical activity score, while FGF23 levels were positively correlated with PTH and creatinine. While SCL did not significantly differ between patients with or without prevalent fractures or MVFs, a significant association was observed between FGF23 and prevalent nonvertebral fractures ($P<0.005$) but not MVFs. Either SCL or FGF23 levels progressively increased with the increase of comorbid conditions, particularly in subjects with the highest severity for CVD, DM and CRF (as assessed by the ICED score). After exclusion of subjects with all those comorbid conditions, the association between FGF23 levels and fractures became nonsignificant. Conversely, a significant association was observed between SCL and MVF ($P<0.01$) when patients with the above cardiometabolic-disorders were considered. Remarkably, either SCL or FGF23 were detected by immunohistochemistry in carotid plaques in most samples, and their expression levels correlated negatively and positively with calcification score, respectively. In conclusion, the relationship between circulating SCL or FGF23 levels and fractures is complex and differ in patients with or without major cardiovascular and/or metabolic conditions. Despite their measurement remains of limited value in clinical practice, a better understanding of the mechanisms underlying their increase in DM and CVD is necessary.

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Gender-different relationship between body composition and incident fracture risk in Koreans: a community-dwelling prospective cohort study

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Low body mass index (BMI) or body weight is a well-known risk factor for osteoporosis and fragility fractures. However, the relative contribution of lean mass and fat mass on bone health, i.e. fragility fractures is inconclusive.
We elucidated the relative contribution of lean and fat mass on fracture risk by group analysis in Korean men and women. This was an ongoing prospective community-dwelling cohort study at Ansung in Korea, begun in 2001. We included 2189 men and 2625 women aged over 40 years. Lean and fat mass was measured using bioelectrical impedance analysis. Study subjects were classified into four groups; non-sarcopenic and non-obese, sarcopenic, obese, sarcopenic obese group according to the gender-specific criteria. Clinical fracture events were assessed at baseline and biennially using a self-reported questionnaires.
During a median follow-up duration of 9.4 years, 77 (3.6%) men and 203 (8.4%) women experienced at least one incident fracture. In cox proportional hazard models for fragility fractures, sarcopenic men had a higher risk for fragility fractures than normal ones even after adjusted for age, height, physical activity,

speed of sound at radius, regular exercise, a history of smoking, a history of drinking, chronic diseases, family history of fracture, and previous history of fracture (hazard ratio (HR) = 2.45, 95% CI = 1.29–4.63), Obese (HR = 0.41, 95% CI = 0.16–1.04) or sarcopenic obese men (HR = 0.2.47, 95% CI = 0.32–18.9) did not have increased risk for fragility fractures compared with normal ones. On the other hand, only sarcopenic obese women had a higher risk for fragility fracture than normal ones after adjustment for covariates (HR = 6.75, 95% CI = 2.68–17.0). Sarcopenic (HR = 1.11, 95% CI = 0.71–1.72) or obese (HR = 1.23, 95% CI = 0.82–1.85) women were not at higher risk for fractures than normal group. We demonstrated the gender-different association between body composition and fracture risk in Koreans aged over 40 years for 10 years. Strengthening muscle mass in men and simultaneous reducing excess fat mass in women is vital in keeping bone health and preventing fragility fractures in Koreans.

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Osteoporosis-related knowledge and health beliefs among female community leaders in Peru

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Background
Postmenopausal women are at high risk for osteoporosis, and early adoption of osteoporosis-preventative behaviours, such as physical activity and dietary calcium intake, can help mitigate this risk. Behavioural studies have shown that knowledge and health beliefs are key factors associated with adoption of healthy behaviours. There are few such studies regarding osteoporosis in South America. Our aim was to conduct an exploratory study evaluating osteoporosis-related knowledge and health beliefs among a group of female community leaders in Peru, who may potentially serve as promoters of bone health in future community-based osteoporosis interventions.
Methodology
We conducted a cross-sectional study among female community leaders in a peri-urban setting. Participants completed a four-part questionnaire that included the internationally validated Osteoporosis Knowledge Test (OKT) and Osteoporosis Health Belief Scale (OHBS), questions regarding sociodemographic and clinical characteristics, and questions pertaining to osteoporosis and fracture risk.
Results
A total of 60 women were interviewed (88% participation rate). Mean age of the participants was 43.7 ± 8.3 years, mean BMI was 30.4 ± 5.3 kg/m^2 and 58.3% had completed high school education or beyond. The majority of interviewed women had a relatively high knowledge regarding osteoporosis based upon the OKT, and reported high perceived benefits to exercise and calcium intake, and relatively high health motivation. The level of osteoporosis knowledge was highly associated with level of education (PR 1.94, 95% CI: 1.23–3.09; $P = 0.005$). We also found a trend for association between level of knowledge and perceived benefits to exercise and calcium intake.
Conclusions
We found that Peruvian female community leaders were knowledgeable regarding osteoporosis and reported high health motivation. Due to their investment in their communities, this population should be a key component of future osteoporosis-related community-based studies and interventions. Finally, we were able to demonstrate that a larger study would be feasible and even desired within this population.

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Incidence of hip fracture in 2010 in Lithuanian residents over 40 years of age

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Objective
To assess the incidence and distribution of hip fractures by sex and age among individuals over 40 years in Lithuania in 2010.
Materials and methods
This population-based study was performed collecting the data from all orthopaedic-traumatology inpatient departments in Lithuania. The case histories of Lithuanian residents over 40 years, who had suffered a hip fracture in 2010, were examined. Subjects with primary hip fracture (ICD-10 codes S72.0, S72.1 and S72.2) were included. The incidence was calculated using the population data of Lithuania in 2010.
Results
Among 2518 subjects (76.4 ± 11.64 years) included, 741 (29.4%) were men and 1777 (70.6%) women, resulting in a female to male rate ratio of 2.4. An average age was 68.8 ± 13.3 in men and 79.2 ± 9.6 in women. Hip fracture incidence was 111.4 per 100.000 men and 192.9 per 100.000 women. Among men the largest group (15%) were aged 70–74 years, and among women 26.9% of fractures occurred at the age of 80–84 years. After the age 75, more women (76.1%) than men (40.8%) had suffered a hip fracture ($P < 0.001$). There were no differences between men and women in the frequencies of left-sided and right-sided fractures as well as in fracture type. Two thirds of subjects (66.3%) were urban residents. No significant differences in hip fracture incidence and average age of fracture were found between urban and rural residents. However, at the age 75 and more, the fracture incidence was higher in urban than in rural residents (685 and 555/100.000, respectively; $P < 0.001$).
Conclusions
In Lithuania in the year 2010, hip fracture incidence was 111.4 per 100.000 men and 192.9 per 100.000 women above the age of 40 years. In the age group of 75 and above, the higher incidence of hip fracture was found in urban than in rural residents.

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Seasonal and environmental effect on the incidence of hip fractures and non hip fractures in Italy

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Background and objective
Data concerning the incidence of fractures in Italy have been displayed since year 2000 by analysing hospital discharge records (HDRs). However, the influence of seasonal and environmental factors on fracture events in the Italian population has not yet been assessed.
Methods
We have analyzed HDRs for primary diagnosis of hip, vertebral, forearm, humeral and knee fractures in Italy from year 2007 to 2009. Inferential statistics have been performed to test the influence of the different seasons on the incidence of hip and non-hip fractures, taking into account also the region where fractured people lived, both for men and women.
Results
Both the incidence hip and non-hip fractures in winter and autumn is significantly higher than that observed in spring and summer. The incidence of hip and non-hip fractures is higher in Northern Italian Regions than Central and Southern both in males and females.
Conclusion
Our analyses show that the incidence of hip and non-hip fractures is influenced by seasons in a statistically significant way, being higher in winter and autumn. This finding could explain why the incidence of both hip and non-hip fractures is higher in northern Italian regions than in southern and central ones.

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Sex hormone-binding globulin is associated with markers of vertebral fracture and vertebral fracture risk

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Background
The relation between sex hormone-binding globulin (SHBG) and vertebral fracture (VFx) is unclear.
Aim
To examine whether SHBG is associated with bone mineral density at lumbar spine (LS-BMD), trabecular bone score (TBS) and prevalent VFx.
Methods
Data of 6224 men and women participants in the third visit of the first cohort (I-3) and the first visit of the second cohort (II-1) of a prospective population based cohort, were available. Serum SHBG and prevalent VFx were assessed at visit I-3 and II-2, whereas LS-BMD and TBS were assessed 4 years later (visits I-4 and II-2). VFx were scored using the Quantitative Morphometry method (QM) and Algorithm Based Qualitative method (ABQ). Multivariate linear and logistic regression models were performed, adjusted for confounding factors such as medication use, lifestyle factors, body mass index, serum glucose, insulin, calcium and phosphate levels.
Results
We identified 854 prevalent VFxQM and 176 prevalent VFxABQ. After correcting for confounders, higher levels of SHBG were associated with lower LS-BMD (3rd tertile vs. 1st tertile: β: −0.04; 95% CIs = −0.06; −0.02) and higher TBS (third tertile vs first tertile: β: 0.02; 95% CIs = 0.01; 0.03). Higher levels of SHBG were positively associated with both VFxQM (third tertile vs first tertile: OR: 1.25; 95% CIs = 1.02; 1.55) and VFxABQ (third tertile vs first tertile: OR: 2.11; 95% CIs = 1.29; 3.5) independent of BMD and TBS. Adjustment for total testosterone and estradiol levels did not affect any of these associations. Also, no sex differences were observed.
Conclusion
This study suggests that SHBG concentration may be a sensitive and early biomarker of VFx. As measurement of serum SHBG is reliable, easy and inexpensive, its assessment may have clinical utility in identifying individuals at high risk of developing VFx later in life that could benefit from effective preventive interventions.

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The association between diabetes, trabecular bone score, bone mineral density and vertebral fractures

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Background
Fracture risk is increased in Type 2 diabetes (T2D) individuals. Bone mineral density (BMD) is inversely associated with fracture risk but paradoxically high in T2D individuals. Trabecular bone score (TBS) reflects bone microarchitecture and predicts fracture risk.
Objective
We aimed to compare mean lumbar spine TBS (LS-TBS) and lumbar spine BMD (LS-BMD) values, across individuals with and without i) vertebral fracture (VFx), ii) T2D; and iii) estimate the relation between T2D and VFx risk.
Methods
Our study is embedded within a prospective cohort, among subjects aged ≥55 years. LS-TBS and LS-BMD were derived from dual energy X-ray absorptiometry scans (DEXA) whereas vertebral fractures from X-ray measurements. VFx were scored using the quantitative morphometry method (QM) and algorithm-based qualitative method (ABQ). T2D was defined as fasting serum glucose levels higher than 7.0 mmol/l or being on antidiabetic treatment. Multivariate linear and logistic regression models adjusted for age, sex, height and body mass index were used.
Results
Among 4062 participants included in our study, 513 were classified as T2D and 744 had prevalent VFx. Both LS-TBS and LS-BMD were negatively associated with VFx. LS-TBS (OR = 0.113, 95% CI 0.045–0.28, $P < 0.001$) and LS-BMD (OR = 0.46, CI 0.24 0.68, $P = 0.005$). T2D was negatively associated with LS-TBS ($\beta = -0.063$, CI −0.034 to −0.0110, $P < 0.001$) whereas it was positively associated with LS-BMD ($\beta = 0.07$, CI 0.025–0.064, $P < 0.001$). There was no association between T2D and vertebral fracture risk (OR = 1.067 CI 0.828–1.375, $P = 0.61$).
Conclusion
LS-TBS was positively associated with T2D and negatively associated with VFx, despite a higher BMD in diabetics. Our results suggest that LS-TBS may be an indicator of fracture risk despite the higher BMD values in individuals with T2D.

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Long term follow-up of fracture incidence and fracture prediction from bone mineral density

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Introduction
Due to the 'greying' of (industrialized) societies the incidence of osteoporosis and fragility fractures is expected to be increasing. Our aim was to investigate in an elderly population if the incidence of non-vertebral fractures (overall and site specific) has changed after a longer follow-up, and its relation with osteoporosis or osteopenia assessed by bone mineral density (BMD) at baseline.
Methods
Incident non-vertebral fractures were assessed in 14 619 men and women, age ≥45, participants of a prospective cohort study, during a median follow-up of 11.7 (S.D. ±6.2) years (median follow-up: round-1 = 14.5, round-2 = 12.0, round-3 = 5.6 years). Baseline femoral neck BMD was measured and gender specific *T*-scores (NHANES) were calculated. Age adjusted hazard ratios (HR) per SD decrease in BMD were estimated from Cox regression models.
Results
In total, 3981 fractures were observed of which hip (21.3%), wrist (19.3%) and proximal humerus (9.1%) fractures were the most frequent in men and women. Incidence rates per 1000 person-years for all fractures were 21.4 and 5.4, 5.0 and 2.3 for hip, wrist and humerus, respectively. While all- and site-specific fracture incidence was higher in women (and all increased exponentially with age) the HR per SD decrease in BMD was 1.5 (95% CI 1.4–1.6) in women and 1.4 (95% CI 1.3–1.5) in men for all-type of fractures and 2.5 (95% CI 2.0–3.0) in men and 2.1 (95% CI 1.9–2.4) in women for hip fractures. The majority (85%) of non-vertebral fractures occurred above the osteoporosis threshold (*T*-score < −2.5). As compared to individuals with normal BMD levels, individuals with osteoporosis had two-fold higher incidence rate and two- to three-fold higher fracture risk.
Conclusion
After longer follow-up the incidence of fracture seems to remain unchanged with a large fraction of the population fracturing above the BMD threshold of osteoporosis. Combining additional fracture risk assessment tools with BMD remains a need in order to facilitate the implementation of preventative strategies that can decrease fracture incidence.

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Neuropathic pain component in patients with osteoporosis and low back pain

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The aim of this study was to estimate the structure of pain syndrome and reveal the presence of neuropathic pain component in patients suffering from the osteoporosis and low back pain.

Material and methods
We have examined 107 patients aged 45–89 years (average age 68.1 ± 1.2 years). Patients were divided into 2 groups: A – patients with osteoporosis ($n=49$), B – patients with low back pain ($n=58$). And according to age into next groups: 45–60, 61–74, 75–89 years. To assess the NP component, we used painDETECT, LANSS, DN4 questionnaires. To assess intensity of pain, visual analogue scale (VAS) was used. Patients completed Oswestry and Rolland-Morris Disability Questionnaires. For statistical analysis of results, ANOVA, correlation and regression analysis were applied.
Results
Regression analysis shows correlation between the questionnaires: LANSS and painDETECT ($r=0.74$, $P<0.001$), DN4 and painDETECT ($r=0.8$, $P<0.001$). It was found correlation between the visual analogue scale (VAS) and screening scales of neuropathic pain: painDETECT and VAS ($r=0.4$; $P<0.001$). LANSS and VAS ($r=0.3$ $P<0.001$), DN4 and VAS ($r=0.3$; $P<0.001$). 79.6% of patients with osteoporosis examined by painDETECT were unlikely to have the NP component, 14.3% might possibly, 6.1% – probably. LANSS scale: 14.3% were probably to have NP. DN4 scale: 24.5% probably had NP. 58.7% of patients with low back pain examined by painDETECT were unlikely to have NP, 24.1% might possibly, 17.2% – probably. LANSS scale: 24.1% were probably to have NP. DN4 scale: 44.8% had probably NP. In patients with low back pain it was found significant correlation between intensity of pain measured by VAS and Oswestry Disability Index ($r=0.7$, $P<0.001$); between VAS and Rolland-Morris Disability Questionnaire ($r=0.6$, $P<0.001$). Significant correlations were found between Oswestry Disability Index and painDETECT screening scale data ($r=0.4$, $P<0.05$).
Conclusion
In patients with osteoporosis and low back pain the pain syndrome may include NP features. Identification of these would promote a treatment strategy targeted at the NP.

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Tumor necrosis factor superfamily members in bone loss in men with end-stage chronic obstructive lung disease

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Aim
To assess the relationships of serum osteoprotegerin (OPG), receptor-activator of nuclear factor-κB ligand (RANKL) and tumor necrosis factor-alpha (TNF-α) superfamily with bone composition in end-stage COPD.
Methods
Body and bone composition, serum OPG, RANKL, TNF-α and its receptors (sTNFR I, sTNFR II), TNF-related apoptosis-inducing ligand (sTRAIL) levels were measured in 48 men end-stage COPD accepted for lung transplantation, and 36 male non COPD volunteers.
Results
OPG was lower in male COPD patients than in control whereas RANKL, TNF-α and its receptors were higher. No notably difference in the serum sTRAIL concentrations between the two groups. OPG directly correlated with FEV1% ($r=0.49$, $P=0.0005$), fat mass index ($r=0.46$, $P=0.001$), lumbar and femoral T-score ($r=0.653$, $P<0.0001$ and $r=0.686$, $P<0.0001$). Serum RANKL inversely associated with FEV% ($r=0.49$, $P=0.0004$), body lean mass ($r=-0.68$, $P<0.0001$), lumbar and femoral T-score ($r=-0.65$, $P<0.0001$ and $r=-0.56$, $P<0.0001$) but directly correlated with TNF-a ($r=0.52$, $P=0.0002$). A similar pattern of association with FEV1 was observed for sTRAIL, TNF-α and its receptors. OPG was inversely correlated with RANKL ($r=-0.56$, $P<0.0001$), TNF-a ($r=-0.62$, $P<0,0001$), sTRAIL ($r=-0.31$, $P=0.034$) and sTNFR-I ($r=-0.512$, $P<0.0001$). Using backward selection multivariable regression, increased serum TNF-α and RANKL were independently associated with lumbar bone loss (adjusted $R^2=0.61$) while RANKL and weight independently predicted femoral T-score (adjusted $R^2=0.564$). In addition, an increased level of serum RANKL and lowered serum OPG concentration were independently associated with reduced skeletal lean mass (adjusted $R^2=0.51$).
Conclusion
Our results suggest that serum RANKL levels are remained significantly associated with reduced pretransplant BMD in male COPD.

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Keywords: osteoporosis, chronic obstructive pulmonary disease, TNF-α, osteoprotegerin, RANKL, bone metabolism
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Abstract withdrawn.

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Bone mineral density and associated risk factors: in healthy Indian population

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Introduction
Osteoporosis is a global public health problem affecting more than 200 million people worldwide. Osteoporosis has clinical and public health implications because of the mortality, morbidity, and cost of medical care associated with osteoporotic fractures. The World Health Organization (WHO) has defined criteria for assessing bone status by DEXA, which are defined by the T-score, which is the number of standard deviations (SDs) by which a patient's test result exceeds (positive T-score) or falls below (negative T-score) the mean value expected in young healthy individuals. Epidemiology of osteoporosis varies based on risk factors prevalent in a particular geography e.g., dietary habits, socio-economic pattern such as parda custom, exposure to sun, alcohol drinking, smoking habits etc. An understanding of bone mineral density pattern and associated risk factors is crucial for prevention, diagnosis of osteoporosis and management of its complications in later life. Better understanding of known and novel risk factors is expected to improve the decision taken by physician in the prevention and management of osteoporosis.
Methods
This research involves retrospective data collection, from the Preventive Health Check up Department at Max Superspeciality Hospital, Saket, New Delhi, India, over a period of 1 year (2014–2015). The classification for status of BMD has been done based on WHO criteria i.e., normal BMD (T score ≥ -1), osteopenia (T-score < -1 but > -2.5) and osteoporosis (T score ≤ -2.5). The data was collected in structured questionnaire and then recorded in Excel file. Statistical Analysis has partially been done using SPSS version 16.0.
Results
The analyzed population included 57% males; age range: 20–85 years and 43% females; age range: 21–79 years. As per preliminary analysis, osteoporosis has been observed in 7.7, 5.4, 5.4, 3.2 and 4.5% subjects at lumber spine (L1-L4), femur neck (left), femur neck (right), total femur (left) and total femur (right) respectively. Osteopenia has been observed in 31.7, 34.8, 32.6, 30.8 and 28.5% subjects at lumber spine (L1-L4), femur neck (left), femur neck (right), total femur (left) and total femur (right) respectively. Prevalence of osteoporosis increased in this population as the age progressed: <40 years (0%), ≥ 40 years (10%), ≥ 50 years (13%), ≥ 60 years (16%) and ≥ 70 years (17%). In gender-wise analysis for left femur neck bone status, more females reported low BMD (osteoporosis + osteopenia) as compared to males (F vs M: 48% vs 35%). This is to reiterate that results are preliminary only based on unpublished data.

Conclusion
Taking the aforementioned into consideration, an exploratory research has been initiated aimed to analyze osteoporosis disease in adult urban population and to identify risk factors influencing bone mineral density. Further data collection on BMD and risk factors is currently ongoing and will be presented at the Conference.

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Osteoporosis: treatment

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Clinical features of atypical femur fracture

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Introduction
The correlation between the occurrence of atypical femur fractures (AFFs) and prolonged bisphosphonate use has been reported in many studies. However, the incidence of AFFs is extremely low, which results in the lack of studies illuminating its treatment and clinical results so far. In this study, we aim to elucidate the clinical outcomes of bisphosphonate-associated AFFs and the clinical results depending on the bisphosphonate therapy period.
Materials and methods
1. Materials
From 2004 to 2014, we retrospectively reviewed 15 patients (16 cases) with AFFs, of whom four patients had one side complete AFFs and other side incomplete AFFS and one patient with bilateral complete AFFs was considered as two cases. Sixteen typical fracture patients with similar sex, age, injured site, BMI and operation method were used as a control group. Patients with any co-morbidities that may influence bone hearing were excluded from the control group. In one patient, AFF occurred 7 years in right femur after a typical fracture occurred in her left femur and her left typical femur was included in the control group.
2. Methods
We evaluated bone union and complications using radiography and physical examination. Bone union was defined when bone continuity of the fracture site was confirmed in anterior, posterior, and lateral radiography or the maturation of callus was closed in three or more fracture planes out of four without tenderness and movement of the lesions clinically. Use or no use of bisphosphonate, duration and kinds of bisphosphonate were investigated. We compared bone union period and complications with the control group. We evaluated correlation between bone union period and duration of bisphosphonate. The minimum follow up duration was 12 months.

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Postmenopausal osteoporosis tolerance and efficacy of different therapeutic protocols

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Introduction
Osteoporosis is the most common bone disease embrittling. Prevention and treatment strategies are well defined and are always updated. The therapeutic decision is based on the individual risk of fracture, effectiveness and therapeutic tolerance. However, side effects attributed to treatment may exist. Thus, the benefit and risks of prescription drugs is optimized by the choice of the right time and the right treatment.
The objective of the study: Evaluation of the safety and effectiveness of osteoporosis treatments referred in 70 patients followed for postmenopausal osteoporosis.
Materials and methods
Descriptive study conducted with 70 patients followed for postmenopausal osteoporosis. The inclusion criterion was any postmenopausal woman with osteoporosis densitometry objectified by the same apparatus of the dual energy X-ray bone densitometry (Hologic). Were excluded those with secondary osteoporosis, densitometry osteoporosis before menopause, and those with disturbance of calcium and phosphate, factors that may influence bone metabolism and BMD results.
Were identified clinical and laboratory data, supplemented by BMD values (BMD) at the beginning and the middle of the first therapeutic sequence.
The variables studied were the tolerance of different therapeutic protocols referred to bone and their effectiveness in terms of BMD gains between the beginning and the middle of the first therapeutic sequence. The analytical study of values was conducted using the statistical tests student.
Results
They were 70 patients. The average age was 63 years with a standard deviation of 9,19. The average weight was 69 kg (s.d. = 8.94). 75.70% were postmenopausal before age 50 years, beyond the other cases. Nine had a history of fracture with minimal trauma and eight cases among a first degree relative. Eight cases reported the presence of a family osteoporosis. The mean bone mineral density (BMD) values at the entrance of the study were: lumbar spine (2.68 ± 0.64), femoral neck (-1.94 ± 0.78), total hip (-2.12 ± 0.99), forearm (-2.48 ± 2.03).
Blood calcium and phosphate and uriaire was normal (mean serum calcium was 93 mg/l, serum phosphorus in 37.66 mg/l, 24 h urinary calcium 125 mg/24 h, 25 (OH) Vitamin D to 25 ng/ml and PTH in 45 ng/ml (12.54). The majority of patients was under bisphosphonates (alendronic acid as 48.60, 24.30 and 4.30% risedronate as zoledronic acid). 17.10% were under strontium ranelate, 4.30 and 1.40% in the denosumab as Raloxifene. Four patients reported having previously received hormonal treatment of menopause. The median duration of treatment was 24 months (10.7 and 51 months). Regarding side effects with treatment, there were 33.33% in strontium ranelate and 5.88% by bisphosphonates. There was a densitometric gain at three sites significant at the lumbar spine (0.049) and the forearm (0.095).
Discussion and conclusion
The main objective of the current recommendations for the treatment of osteoporosis is to prevent the occurrence or recurrence of fractures. The therapeutic decision is based on the individual risk of fracture,efficacy and degree of therapeutic tolerance. Few studies have compared the different molecules together. Our study showed the effectiveness of different treatments referred to bone densitometry with dice gain the middle of the therapeutic sequence, during the postmenopausal osteoporosis. Few side effects attributed to treatment were highlighted.

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Treatment outcomes during teriparatide use in Greece: country sub-analysis of the Exfos Observational Study

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Scope
Extended Forsteo Observational Study (ExFOS), a multinational, non-interventional, prospective, observational study aims to evaluate fracture outcomes, back pain (BP), compliance and health-related Quality of Life (QoL) in osteoporotic patients prescribed teriparatide (TPTD). We present treatment outcomes for patients (male or female) treated for up to 24 months in Greece.
Methods
Of the 440 patients enrolled in the study 416 (mean age 69.9 ± 9.7 years) have performed at least one active treatment post-baseline visit. These were predominantly women (92.1%), at postmenopausal stage (99.7%). Self-reported parameters of health perception have been recorded throughout the study through validated questionnaires (EQ-5D). We report the observed percent and mean responses.
Results
Seventeen (4.1%) patients had an incident clinical *fracture* during follow-up. *Adherence:* 80% of subjects used the treatment through month 23; 45% continued use through month 24. *BMD* numerically increased in all sites. Indicatively, Lumbar BMD *T*-score (mean (s.d.)) increased from -3.39 (0.73) at baseline to -2.36 (0.63)

at study end. *BP* history the last year before enrollment was reported by 88.9%. During treatment, all self-reported variables improved. Examples of BP frequency (% of patients with BP fairly often or almost daily) and BP severity (% with moderate-severe BP), EQ-5D Visual Analogue Scale (VAS) score and EQ-5D mobility (% reporting some or extreme problems) are depicted below.

	Baseline	3 m	6 m	12 m	18 m	24 m
BP frequency (%)	68.2	33.5	22.6	14.1	17	11.6
BP severity (%)	76.2	50.4	32.8	20.9	22.2	18.6
EQ-5D VAS	57.4	66.2	71.3	75.4	76.7	83
EQ-5D Mobility (%)	57.9	39.0	29.9	22.5	17.7	16.2

Conclusions
Use of TPTD remains high almost throughout the follow-up. A decrease at month 24 may be due to prescribing (24 months/26 cartridges) particularities. Patients experience improvement in BP and QoL. Fracture rate was low. Results should be interpreted in the context of an observational study.

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Effects of single bolus injection of vitamin D on serum FGF23, Sclerostin and DKK1 concentrations in vitamin D-deficient subjects

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Objective
Bone is one of endocrine organ and osteocytes secretes FGF23, Sclerostin and DKK1. FGF23 inhibits renal phosphate reabsorption and suppresses 1-α hydroxylase activity. Calcitriol stimulates FGF23 synthesis in bone. We aimed to determine the effect of single bolus injection of vitamin D on serum FGF23, DKK1 and Sclerostin concentrations in vitamin D-deficient subjects.
Design and methods
The study group was composed of placebo group with severe vitamin D deficient group ($n=9$, 25 (OH) D $=8.77\pm0.73$, mean age 30.56 ± 2.63 years), intervention group with severe vitamin D-deficient healthy subjects ($n=10$, 25(OH) D $=7.56\pm0.48$ ng/ml, mean age 31.80 ± 2.51 years), intervention group with vitamin D-deficient healthy subjects ($n=11$, 25(OH) D $=13.57\pm0.76$, mean age 35.55 ± 2.52 years). The groups were compared for serum FGF23, Sclerostin, DKK1, intact parathyroid hormone (PTH), and urinary excretion of calcium and phosphate after single injection of plain vitamin D 20,000 unit.
Results
Serum 25(OH)D was increased significantly 1, 2, 3 and 3.5 month after single bolus injection of 20,000 unit of vitamin D, however intact PTH was not changed significantly after treatment. Serum FGF23 concentrations were not significantly changed after treatment in both intervention groups. Serum FGF23 was slightly elevated in two subjects with IDA with iron replacement treatment. Serum Sclerostin was also decreased slightly after replacement, however, serum DKK1 was not changed significantly at all.
Conclusion
Single bolus injection of vitamin D does not show any significant change of serum FGF23, Sclerostin and DKK1 concentrations and it may not have unfavorable effects on bone formation and mineralization.

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A interventional study in a real life setting to assess the clinical efficacy and effect to fracture in the 1 year after the injection of zoledronic acid in osteoporotic patients with long bone or spine, pelvic fractures

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Objectives
There are some studies that evaluated changes of bone marrow density (BMD) and re-fracture after zoledronic acid injection. However, there is no study that showed about the fracture on all of the body, fracture healing, and improvement of clinical symptom after zoledronic acid injection. In this study, authors evaluated the 1 year BMD change, changes of lumbar pain in lumbar spine fractures, effects on fracture healing, re-fracture and additional fracture on other site after zoledronic acid injection in patients with spine and non-spine fracture.
Methods
Patients who had lower than -2.5 (*T*-score) at BMD from January 2011 to June 2012 in our hospital were evaluated. Among them, patients who had spine fracture and/or non-spine fracture were enrolled. Zoledronic acid was injected in 3 days after fracture. BMD was checked 1 year later after injection. Furthermore, changes of lumbar pain in lumbar spine fractures was evaluated every weeks, effects on fracture healing, re-fracture and additional fracture on other site also were evaluated for this study.
Results
Spine fracture group ($n=97$) and non-spine fracture group ($n=31$) showed significant increasing of BMD ($P<0.05$). Visual analogue scale (VAS) for lumbar pain after spine fracture was reduced from 6.6 ± 2.2 before zoledronic acid injection to 4.2 ± 1.1 after injection. There was no delayed union or non-union after non-spine fractures. However, new fractures developed in three cases (2.34%); two spine fractures, one distal radius fracture.
Conclusions
There are increasing of BMD, relief of lumbar pain, no disruption to fracture healing after injection of zoledronic acid for osteoporotic patients who has spine fracture or non-spine fracture is good treatment option.

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Magnesium together with Calsium and vitamin D improves the bone metabolism in (70y) healtly females

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Background
In the National Osteoporosis Guide 2014 all the patients are first recommended adequate calcium and vitamin D intake. For additional treatment US FDA approves the use of medications within seven different drug groups, but no magnesium. We have therefore studied the beneficial effect of Mg added to a Ca/vitamin D regime.
Study
Fifty healthy women aged above 70 years recruited from elderly center were randomized into a Mg supplementation group (Mg+) and a control group (Mg−). They were all given for 28 days twice daily tablets with calcium 500 mg and vitamin D_3 5 µg Weifa. In the (Mg+) group they received Mg 120 mg Takedanycomed x 2 and in the (Mg−) group placebo x 2. They were seen at day 0, day 7 and day 28 with blood sampling at the same time of the day. The study was accepted by the Regional etical commitee ref. 584-05-99010, Clinical Trials gov Registration NCT 02549521.
Results
The concentration differences (Mean $\pm$ S.D.) from 0 to 28 days were for Mg supplemented ($n=23$), and placebo ($n=22$) respectively: S-Mg (mmol) 0.04 ± 0.05**, 0.02 ± 0.05*; Ratio U-Ca/creatinine (µmol/µmol) 0.11 ± 0.15¶¶, and 0.10 ± 0.25; Ratio U-Mg/creatinine (µmol/µmol) 0.12 ± 0.14¶¶ and -0.02 ± 0.12##; PTH (pmol/l) -0.88 ± 1.65*, and 0.55 ± 1.83; S-Bone ALP (µ/l) 0.25 ± 3.53, and -0.86 ± 4.46; S-Osteocalcin (nmol/l) 0.46 ± 0.39¶¶ and 0.12 ± 0.12; S-1-CTP (µg/l) 0.10 ± 0.61 and -0.09 ± 0.45; U-PYD/creatinine (nmol/mmol) -1.52 ± 3.55¶ and -1.76 ± 4.97; U-NTx/creatinine (nmolBCE/mmol/l creatinine) -19.1 ± 32.0¶¶ and -32.5 ± 38.0.#. Parret t-test $P\leq0.05$*, $P\leq0.01$**. Wilcoxon sign rank test $P\leq0.05$¶, $P\leq0.01$¶¶. Comparison between groups Mann–Whitney U-test, $P\leq0.05$ #, $P<0.01$##.
Conclusion
From the point of calcium and bone metabolism these results show the benefit of Mg supplementation in addition to supplementation with Ca and vitamin D. Patent pending.

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The effect of teriparatide on fracture healing of vertevral compression fracture in postmenopausal women

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Introduction
Acute vertebral compression fractures cause severe back pain and need long time to heal. The progression of fracture or nonunion is not rare. The teriparatide is a

synthetic parathyroid hormone which has been used as anabolic agent and treatment of osteoporosis. It can also be used for promoting fracture healing in special condition. Periodic infusion of teriparatide enhances bone formation and increases bone strength. We evaluated the effect of periodic teriparatide infusion on fracture healing of acute vertebral compression fractures.
Methods
A prospective study consisting of a review of case report form. We prospectively enrolled 84 postmenopausal women who had one or two acute painful vertebral compression fractures confirmed by MRI. All patients were treated conservatively. Among them, 32 patients were treated conservatively with teriparatide for at least 6 months (group I), and 52 were treated with antiresorptive agent (group II). VAS and ODI scores were assessed at each follow up until 1 year after trauma. We also measured radiographic changes, the degree of collapse progression.
Results
The progression of fractured vertebral body collapse was shown in both groups, but the degree of progression was significantly lower in group I than in group II. At the last follow-up, mean increments of kyphosis and wedge angle were significantly lower in group I. Clinical outcome measures were closely related with vertebral body collapse.
Conclusion
Periodic infusion of teriparatide might have many beneficial effects on acute - vertebral compression fractures in postmenopausal women. Patients using - teriparatide showed quicker symptomatic improvement and less collapsed vertebral body 1 year after trauma compared to patients using anti-resorptive agent.

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Atypical femoral fractures after long-term bisphosphonates therapy: case report

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Introduction
Bisphosphonates are the most commonly prescribed type of medication for the treatment of osteoporosis. This treatment, however, is not without adverse effects. Several case reports and case series have indicated an association between a unique fracture type, so-called 'atypical femoral fractures,' (AFF) and prolonged bisphosphonate use.
Case report
We present a 77-year-old woman with no history of trauma, or associated with low-energy trauma, admitted to our clinic after three weeks of a left femoral fracture threated in Orthopedic Clinic. The patient was in our clinical observation since 1983 for hypothyroidism after thyroidectomy in treatment with 100 mcg LT4. In 2002, the patient was diagnosed with osteoporosis following the DXA examination. The bone mineral density (BMD) showed *T*-score at total lumbar spine: −4.0, total hip: −2.1, and left femur neck: −3.0. 25-OHD3 level varied between 17.69 and 5.54 ng/ml. TSH, FT4, and FT3 level was normal in time. Has been established treatment with risedronicum acidum 35 mg/week, Alfacalcidol 1 mcg/day and calcium 1000 mg/day. Annual DXA-BMD shows a slight increase of BMD; patient medical treatment has been changed in zoledronicum acidum and then on alendronicum acidum. The patient was in treatment with bisphosphonates over 10 years. An association between bisphosphonate long-term use and the occurrence of AFF has been suggested. This diagnostic has been supported and by the X-ray: femoral shaft region transverse fracture configurations, absence of comminution, a medial spike, localized periosteal thickening of the lateral cortex, and generalized thickening of the femoral cortices.
Discussion and conclusions
The causal relationship between prolonged bisphosphonate use and the occurrence of AFF has not yet been established. For the patient at high risk of fracture, it may be beneficial to continue bisphosphonate treatment beyond 5 years. For most people with osteoporosis, the proven fragility-fracture risk-reduction benefits of bisphosphonates outweigh the risks of AFF.

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Effect of 61-week administration with beta-hydroxy-beta-methylbutyrate on volumetric bone mineral density of lumbar spine in osteoporotic patient

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Currently available osteoporosis treatment include antiresorptives and/or anabolic agents. Studies in animals have shown that beta-hydroxy-beta-methylbutyrate (HMB) administration affects anabolically bone tissue metabolism and improves morphological, densitometric and mechanical properties of bones in axial and appendicular skeleton. Thus, the aim of the study was to present first data on effects of 61-week treatment with HMB on volumetric bone mineral density (vBMD) of lumbar spine in female patient suffering from postmenopausal osteoporosis. The therapy with calcium salt of HMB (CaHMB – HMB Mega Caps 1250, Olimp Sport Nutrition, Poland) was performed orally (1250 mg/day). One CaHMB capsule consisting of 1000 mg of pure HMB was taken during the diner each day throughout 61 weeks. Quantitative computed tomography method and SOMATOM EMOTION SIEMENS apparatus (Siemens, Erlangen, Germany) equipped with Somaris/5 VB10B software (version B10/2004A) and Osteo CT application package were used to determine vBMD of the trabecular and cortical bone compartments in each lumbar vertebrae (L_1–L_5). Calcium hydroxyapatite (Ca-HA) density of trabecular bone ($Tb_{Ca\text{-}HA}$) was measured in the central part of the cross-section of the vertebral body, while calcium hydroxyapatite density of cortical bone ($Cb_{Ca\text{-}HA}$) was determined on the margins of the vertebral body. The measurements of vBMD were performed at the baseline and 61 weeks later.
61-week long oral administration with CaHMB in the osteoporotic patient increased $Tb_{Ca\text{-}HA}$ and $Cb_{Ca\text{-}HA}$ by 1.98% and 7.99%, respectively. The increased values of *T*-score (20 years) and *Z*-score by 0.07 and 0.12 were also stated as the result of the 14-month therapy with HMB.
In conclusion, this study indicates that HMB improves vBMD of lumbar spine and may be applied for effective treatment of osteoporosis in humans. However, further studies on wider human population are recommended to evaluate dose-related effectiveness and possible mechanisms influencing bone tissue metabolism by HMB.

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AMD3100 improves ovariectomy-induced osteoporosis in mice by facilitating mobilization of hematopoietic stem/progenitor cells

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Inhibition of an increase of osteoclasts has become the most important treatment for osteoporosis. The CXCR4 antagonist, AMD3100, plays an important role in the mobilization of osteoclast precursors within bone marrow (BM). However, the actual therapeutic impact of AMD3100 in osteoporosis has not yet been ascertained. Here we demonstrate the therapeutic effect of AMD3100 in the treatment of ovariectomy-induced osteoporosis in mice. We found that treatment with AMD3100 resulted in direct induction of release of SDF-1 from BM to blood and mobilization of hematopoietic stem/progenitor cells (HSPCs) in an osteoporosis model. AMD3100 prevented bone density loss after ovariectomy by mobilization of HSPCs, suggesting a therapeutic strategy to reduce the number of osteoclasts on bone surfaces. These findings support the hypothesis that treatment with AMD3100 can result in efficient mobilization of HSPCs into blood through direct blockade of the SDF-1/CXCR4 interaction in BM and can be considered as a potential new therapeutic intervention for osteoporosis.

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Hypercalcemia after discontinuation of long-term denosumab treatment

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Purpose
Denosumab is commonly used as an anti-resorptive agent to treat osteoporosis. After discontinuation of denosumab, however, bone resorption increases again, and the bone mass gained during therapy is lost within a year.
Methods
We present a case report of asymptomatic hypoparathyroid hypercalcemia in a patient who discontinued long-term treatment with denosumab.
Results
A 67-year old women with osteoporosis was treated with denosumab 60 mg subcutaneously every 6 months from 2004 to 2014. She received the last injection in May 2014. Routine biochemistry in November 2014 showed increased s-ionized calcium (I-Ca) 1.64 mmol/l (1.18–1.32 mmol/l) and suppressed p-parathyroid hormone (PTH) 1.6 pmol/l (1.6–6.9 pmol/l). The patient was extensively examined, but no underlying disease was found. In January 2015 the patient began treatment with alendronat 70 mg weekly. Highly elevated type 1 collagen C-terminal cross-linked telopeptide, procollagen type 1 N-terminal propeptide and bone-specific alkaline phosphatase were found in April 2015. From then on, I-Ca and PTH normalized and the bone turnover markers (BTM) decreased. (Table 1)

Table 1 The development of bone-related biochemistry from November 2014 to September 2015.

Parameter (reference range)	November 25, 2014	December 22, 2014	January 8, 2015	March 16, 2015	April 1, 2015	May 27, 2015	September 2, 2015
s-I-Ca (mmol/l) (1.18–1.32)		1.64	1.57	1,41	1.29	1.32	1.26
p-PTH (pmol/l) (1.6–6.9)	1.6	1.7		2.5	3.9	4.3	4.2
p-CTX (μg/l) (0.03–0.83)					1.47	1.54	1.12
p-P1NP (μg/l) (13–116)					304	288	203
p-eGFR (ml/min) (>60)	58	52	59	80	74		79

s: serum, p: plasma, I-Ca: ionized calcium, PTH: parathyroid hormone, CTX: C-telopeptide of type I collagen, P1NP: N-terminal propeptide of type 1 procollagen, eGFR: estimated glomerular filtration rate.

Conclusion
In this case report, we describe increased BTMs and hypercalcemia associated with discontinuation of 10 years of treatment with denosumab. The increase in BTMs is assumed to be temporary and normalization is expected. Since denosumab is commonly used, there is an urgent need for evidence-based guidelines on discontinuation of long-term treatment, avoiding side effects and preserving anti-fracture efficacy.

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P390

Denosumab therapy results in a high frequency of responders by bone mineral density in both treatment-naïve patients and patients switching therapies

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Clinical trials suggest that denosumab (DEN) therapy results in greater increases in bone mineral density (BMD) in treatment-naive patients than in patients switched from bisphosphonates.
We retrospectively reviewed charts of all patients treated with DEN at an osteoporosis referral centre in Vancouver, Canada including all patients treated with DEN 60 mg SC every 6 months for 1 year or more, and in whom baseline and follow-up BMDs were available. BMD was followed at a single site, whichever of spine or hip was lower. Patients were either treatment-naive, or switched from alendronate, risedronate, zoledronic acid (ZOL), or teriparatide.
A total of 758 patients were included, 310 followed at hip and 448 followed at spine. All groups increased BMD on DEN. We defined a responder as being a patient with >3% increase in BMD at spine or total hip site at one year. In all groups, 65% of patients responded at spine and 34% responded at hip at one year. Responders increased to 87 and 52% at spine and hip respectively at 4 years. The greatest proportion of responders was in the treatment naïve group where at 1 year, 100% showed a response at spine and 83% showed a response at hip. Compared to other groups, patients switched from ZOL had fewer responders at hip and spine at 1 year and this difference persisted at 3 years at hip.
We observed a greater than expected BMD responder rate in patients switching from bisphosphonate to DEN as compared to published clinical trials. Lower adherence to prior therapy and the use of generic bisphosphonates in clinic patients may account for some of this difference. Our results will inform clinicians regarding the likelihood of seeing a significant BMD response when transitioning individual patients to DEN.

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P391

Prevalence of osteoporosis and effectiveness of screening test using ultrasound bone densitometry and education in a community-dwelling population

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Background
In 2004, we reported a 48.1% prevalence of osteoporosis in an Ibansung cohort. The current prospective intervention study was undertaken in order to estimate the prevalence of osteoporosis from 2004 to 2015 and the increasing treatment rate of osteoporosis following osteoporosis screening tests with ultrasound bone densitometry and education in the same cohort.
Methods
From November 1, 2014 to August 31, 2015, 960 adults ≥50 years of age were enrolled in this study. All participants received screening tests for osteoporosis using ultrasound bone densitometry and education concerning osteoporosis and related conditions. The participants were interviewed using a questionnaire on the diagnosis and initiation of osteoporosis treatment during the follow-up period.
Results
Of 960 potential participants, 865 were included; 88 individuals were not in the Ibansung region at the time of the study and 7 people had died. Of these, 595 (68.8%; 150 men and 445 women) were given bone densitometry measurements and completed the questionnaire. The mean age of the participants was 74.0 years (range, 51–94 years). Of the 595 participants, 393 people (66.1%; 326 woman and 67 men) were diagnosed with osteoporosis (T score < -2.5). The prevalence of osteoporosis showed an increasing trend, from 48.1% in 2004 to 66.1% in 2015. Of the 393 participants diagnosed with osteoporosis, 65 participants received additional bone densitometry measurements while hospitalized and osteoporosis management was re-initiated in 44 patients. The osteoporosis management rate in the study cohort increased from 21.6 to 32.8%, with osteoporosis diagnosed in 66.2% of participants at the latest follow-up.
Conclusions
This prospective intervention study demonstrated that a screening test and an educational brochure increased the treatment rate from 21.6 to 32.8%. Therefore, dissemination of knowledge about osteoporosis can be helpful in increasing the treatment rate of osteoporosis in community-dwelling individuals.

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P392

The effect of oral dabigatran etexilate on bone density, strength, and microstructure in female and male mice

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Pradaxa (dabigatran etexilate) is a new anticoagulant, which recently has been approved for clinical use. Dabigatran etexilate (DE) is a direct thrombin inhibitor, which in addition to its effect on blood clotting, may have an anabolic effect on bone. Recently, it has been reported that DE could significantly reduce bone resorption and enhance bone formation resulting in a significant and substantial increase in trabecular bone mass in mice.
The aim of the present study was to investigate whether DE can increase aBMD, bone strength, and microstructure in male and female mice.
A total of 28 male 14-week-old C57BL/6 mice were randomized by weight into four groups: 1. Control 3 weeks; 2. DE 3 weeks; 3. control 6 weeks; 4. DE 6 weeks. An identical design was applied to 26 female C57BL/6 mice. DE was

given in the chow (15 mg/g chow) and offered *ad libitum*. The animals were killed after 3 or 6 weeks, respectively. aBMD and BMC were evaluated with pDEXA, 3D microstructural properties were determined with μCT, and bone strength were determined with mechanical testing. The experiment was approved by the Danish Animal Experiments Inspectorate.
Significant higher tibial and vertebral aBMD ($P<0.05$) were found in DE-treated female mice, but not in DE-treated male mice after 6 weeks intervention. No significant changes were found in distal femoral epiphyseal or metaphyseal microstructure and vBMD in female or male mice. No significant changes were found in proximal tibial metaphyseal microstructure and vBMD, and no significant changes were found in tibial three-point bending strength and stiffness in female or male mice.
In conclusion, DE increased tibial and vertebral aBMD in female mice, but not in male mice. Furthermore, no effects of DE were found on trabecular bone 3D microstructure and tibial bending strength in either female or male mice.

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P393

Long-term high-dose resveratrol supplementation reduces bone mass and strength in rats

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Background
Resveratrol (RSV), a natural polyphenolic compound, stimulates osteoblasts and inhibits osteoclast activation *in vitro*. Conflicting results have emerged from short-term studies in rodents but a recent study in men suggests a positive effect on BMD.
Aim
To evaluate effects of short- and long-term high-dose RSV supplementation on bone in immobilized and non-immobilized rats.
Methods
A total of 72 female Wistar rats were randomly allocated to six groups. Two baseline groups (BSL_{PD} and BSL_{Rsv}) underwent short-term diet-intervention only, being fed a phytoestrogen-deficient diet (PD) or a RSV diet (600 mg/kg body weight/day) for 4 weeks before sacrifice. Four groups ($IMMOB_{PD}$ and $IMMOB_{Rsv}$, and MOB_{PD} and MOB_{Rsv}) were injected in the right hindlimb with either botulinum toxin (BTX) (immobilized) or saline (non-immobilized), and fed either a PD diet or a RSV diet 4 weeks pre-injection and six weeks post-injection (long-term diet intervention) before sacrifice. DXA, μCT, and biomechanical tests were used for evaluation.
Results
Short-term RSV treatment did not affect the measured bone parameters, whereas long-term RSV exposure had a consistent negative impact on non-immobilized rats (MOB_{Rsv} versus MOB_{PD}) (results presented as mean percent difference with 95% CI); aBMD (−4.6% [−7.95; −1.25]%, $P=0.009$), distal femoral Tb.N (−12.1% [−21.4; −2.9]%, $P=0.01$), Tb.Sp (+17.4% [2.4; 31.9]%, $P=0.03$), and BV/TV (−11.4% [−23.6; 0.9]%, $P=0.07$). In addition, RSV reduced femoral mid-diaphyseal three-point bending strength (−9.1% [−17.0; −1.2]%, $P=0.03$) and stiffness (−8.7% [−17.2; −0.23]%, $P=0.04$). BTX-induced immobilization resulted in significant bone loss and reduced bone strength, and RSV supplementation was unable to prevent this.
Conclusion
Long-term high-dose RSV reduced bone mass and strength and did not prevent immobilization-induced bone loss.

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P394

Inhibitory effects of nanopartical calcium administration on dexamethasone-induced osteoporosis development in axial skeleton of pigs

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The aim of the study was to evaluate effects of 6-month-long administration with nanopartical calcium on morphological and densitometric properties of axial skeleton in pigs subjected to osteoporosis induction with dexamethasone. Male newborn piglets ($N=28$) were divided after birth into four equal groups (control group receiving placebo, Dex group receiving dexamethasone, NanoCa group receiving orally nanopartical calcium and NanoCa/Dex group) receiving simultaneously dexamethasone and nanopartical calcium. At the age of 6 months of life all pigs were sacrificed to obtain the second lumbar vertebra (L2) for analyses. Bone mineral density (BMD) and bone mineral content (BMC) were evaluated with the use of dual-energy X-ray densitometry (DEXA) method and Norland XR-46 apparatus, supplied with Research Scan software (Norland, Fort Atkinson, WI, USA), immediately after record of vertebral weight and vertebral body length (VBL). Statistical analysis of data was performed using one-way ANOVA and multiple comparison *post hoc* Duncan test. $P<0.05$ was considered as statistically significant. Dexamethasone administration significantly decreased VBL, BMD and BMC of L2 ($P<0.05$). Bone weight, VBL and BMC were significantly decreased in NanoCa Group when compared to the controls ($P<0.05$). Nanopartical calcium administration was effective to increase BMD and BMC of L2 in pigs receiving dexamethasone simultaneously ($P=0.02$ and $P=0.01$).
In conclusion, this study has shown positive effects of nanopartical calcium administration on densitometric properties of axial skeleton in pigs long-term treated with dexamethasone. The obtained results indicate that naopartical calcium administration may be effective for prevention and treatment of steroid-induced osteoporosis.

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P395

Acute kidney injury after a single intravenous zoledronic acid administration in patients with osteoporosis

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Safety data on zoledronic acid (ZA) administration for osteoporosis suggest potential kidney toxicity; indeed, it is not indicated in patients with impaired renal function. Patients' clinical monitoring usually includes glomerular filtration rate (GFR) evaluation; no study addressed the issue of the early kidney injury. We evaluated the early (within 3 months) effect of iv ZA on renal function defining the potential role of AKI biomarkers in unveiling subtle damage.
Five mg i.v. ZA infusion was administered for the first time to 23 patients with osteoporosis and normal renal function (17 women and six men, mean age 73 ± 7 s.d. years). AKI biomarkers (urinary (u) NGAL, KIM-1, and MCP-1; serum (s) MCP-1 and IL-18) were assessed at baseline and at day (d) 2 and 30 after administration. Creatinine clearance (CrCl), plasma C-terminal FGF-23, sKlotho, calcium excretion (CaEx) and renal threshold phosphate concentration/GFR ($TmPO_4$/GFR) were also measured.
No significant changes in mean levels of urinary markers were detected. Mean values of sIL-18 and sMCP-1 significantly increased at d 2 (44 ± 88%; $P<0.01$ and 198 ± 237%; $P<0.001$) and returned to baseline levels at d 30. Mean CrCl values did not significantly change at d 30. We observed no difference in mean levels of plasma C-terminal FGF-23 and sKlotho at any time. There was a 28 ± 59 and 26 ± 43% decrease in CaExcr at d 2 ($P<0.05$) and d 30 ($P<0.01$), respectively. $TmPO_4$/GFR significantly decreased at d 2 and d 30 (−8.6 ± 15.9%, $P<0.05$ and −11.3 ± 13.5%, $P<0.001$).
Our data show that there is an acute renal damage as early as 24 h after ZA infusion in osteoporotic patients with normal renal function. Renal injury is apparently reversible after 1 month. Secondary hyperparathyroidism could be responsible for reduction in $TmPO_4$/GFR and calcium excretion.

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P396

Oral health assessment necessary with comprehensive geriatric assessment

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Objective
Good oral health is important for well being especially in the elderly. Osteonecrosis of Jaw is rare side effect of bisphosphonates and Denosumab. Patients need adequate dental precautions and appropriate advice from doctors and dentists to reduce the chance of this condition. Aim of the study was to assess the dental health of patients with osteoporotic fractures receiving bisphosphonate or denosumab treatments.
Methods
Single person administered Questionnaire study.
Subjects
Patients who had suffered fragility fracture and were receiving Bisphosphonate or Denosumab treatment.
Setting
Teaching hospital in United Kingdom.
Analysis
Responses were summarised and percentages calculated.
Results
Age range 65–94 years Females – 74%.
Number of patients studied – 100.
Sixty-six percent patients were registered with a Dentist. Reasons mentioned for those not registered were- not found necessary (30%), cost (35%) availability (35%). Sixty-eight percent visited their dentist twice or more within last 2 years. Sixty-four percent brushed their teeth daily. Eighteen percent had full or partial dentures. Seventy-four percent reported loose or sensitive teeth. Self reported dental health rating was- Good 32%, average 25% and bad 43%. Twenty-three percent had some dental procedure done within last 2 years. Fifty-five percent reported dental/gum infections in last 2 years.
Fifty-four percent reported medical/dental health professional informing side effects and precautions associated with osteoporosis drugs. Ten percent reported having discussion about BRONJ. Fifty-two percent had dental checkup done prior to initiating osteoporosis treatment.
Conclusions
This large study shows poor dental health is common amongst patients receiving osteoporosis treatments. There is a need to monitor, improve and maintain good oral health both by medical and dental professionals. All patients need information, education and advice about risks and benefits associated with osteoporosis therapies. Further large studies are needed to develop and validate a useful and practical Oral health assessment tool. We recommend that 'oral health risk assessment' should be integrated with 'comprehensive Geriatric assessment tools' for elderly patients.

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P397

Evaluating dental health risk assessment amongst bisphosphonate users

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Objective
With increasing usage of bisphosphonates and increasing longevity, many elderly patients are on long term bisphosphonates and may require some dental procedure. We wanted to determine the dental risk status based on Scottish guidance for Dental practitioners 2011.
Methods
An interviewer recorded information needed to classify risk status amongst a randomly selected database of bisphosphonate users.
Setting
Teaching hospital in United Kingdom.
Results
Age range 65–94 years, total number studied = 100. 74% were females.
Seventy-eight percent users were at low risk (taking bisphosphonates for the prevention/management of osteoporosis. Twenty-two percent patients were at higher risk (previous diagnosis of BONJ, taking a bisphosphonate as part of the management of a malignant condition, Paget's disease, osteogenesis imperfecta, systemic corticosteroids, immunosuppressants, coagulopathy, chemotherapy or radiotherapy) for development of rare condition Osteonecrosis of jaw.
Conclusions
This study shows that amongst bisphosphonate users, a significant proportion of elderly are at high risk of developing dental complications following invasive dental procedure. Risk assessment should be performed and documented as a routine in these patients. If any extraction or invasive dental procedure is deemed necessary then especially amongst high risk patients it should be carried out before commencement of bisphosphonate therapy.

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P398

Dementia and hip fractures

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Objective
From April 2012, pre- and post-operative cognitive assessment amongst hip fracture patients is one of the criteria for enhanced payment to hospitals in England in UK. Aim of the study was to assess the prevalence of dementia amongst hip fractures and assessments from specialist mental health services.
Methods
Prospective study.
Setting
Acute hip unit at a UK hospital in Wales(not England).
Subjects
Patients admitted following hip fractures.
A single researcher collected data on the prevalence of dementia and mild cognitive impairment (MCI) amongst hip fracture patients after their surgery just before discharge using MMSE and 6CIT cognitive assessments scales.
Results
Age range 65–94 years 70% females.
Total number of study population = 80.
The prevalence of dementia amongst older hip fracture patients was 26%. The prevalence of mild cognitive impairment was 42%. No significant differences in the prevalence of dementia or cognitive impairment was noted between the sexes. Patients from long-term care setting were significantly more likely to have dementia (49%) compared to those admitted from the community (18%). 40% of patients had a documented past history of dementia and 25% of these were on dementia medications. Despite access to specialist mental health services, only 15% patients were referred/assessed by specialist Mental Liaison team.
Conclusions
A significant proportion of elderly hip fracture patients have cognitive impairment and dementia, many are previously undiagnosed. These patients would benefit from further detailed assessment from LIAISON Psychiatry team following initial diagnosis. Our findings suggest need for improved staff awareness and training to detect dementia early, to ensure that hospitals have a care pathway in place for dementia that fits with existing acute care pathways. Improved diagnosis and management for this high risk group has potential implications in reducing length of stay not only in acute hospital but also the overall burden of dementia after discharge.

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P399

Need for integrated proactive approach for dementia and hip fracture care

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Objective
From April 2012, pre- and post-operative cognitive assessment amongst hip fracture patients is one of the criteria for enhanced payment to hospitals in England in UK. Aim of the study was to assess the association between dementia and hip fractures
Methods
Setting – Acute hip unit at a UK hospital in Wales (not England).
Subjects – patients admitted following hip fractures
A single researcher collected data on the prevalence of dementia based on AMTS amongst hip fracture patients from medical records.
Results age range 65–94 years 70% females.
Total number of study population = 130.
Twenty patients did not have any mental assessments done. The prevalence of dementia amongst 110 hip fracture patients was 26%. Patients from long-term care setting were significantly more likely to have dementia (49%) compared to those admitted from the community (18%). Forty percent of dementia patients had a documented past history of dementia.

Conclusions
Fracture patients have a high prevalence of dementia and cognitive impairment. Many are previously undiagnosed, do not routinely receive cognitive assessment. This is a missed opportunity for the early diagnosis and providing integrated care for patients suffering with dementia. There is a need for good-quality early diagnosis and intervention for all suspected dementia patients- through specialist assessment that delivers rapid assessment, accurate diagnosis and treatment, care and support.

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P400

The upper two third of the reference interval of the serum calcium level is required for the optimal increase in lumbar bone mineral density by bisphosphonate and active vitamin D3 analogue for osteoporosis

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Background
It is important to increase bone mineral density (BMD) by the treatment with bisphosphonates (BPs), as lower BMD has been revealed to be one of the risk factors for the subsequent fragility fracture in patients with osteoporosis treated with BPs.
Methods
The two independent populations of the patients with postmenopausal osteoporosis who were treated for 2y by BPs with active vitamin D analogue (aVD) were enrolled in Study 1 ($n=93$) and Study 2, ($n=99$) in this retrospective study. The most appropriate cut-off level of serum Ca (sCa) for optimal increase in LS-BMD by the combined therapy for six months was calculated using Akaike's information criterion (AIC).
Results
In Study 1, the lumbar spine (LS)-BMD of the patients was significantly increased by the combined therapy for 2y ($P<0.001$, 5.4%). A multiple regression analysis revealed that the sCa was the factor that was significantly associated with the increase in LS -BMD for 2 years (R^2:0.088, $P=0.02$). The sCa of 9.3 mg/dl, which was the borderline between the lower and middle tertiles of its reference range, was found to be a cut-off levels for the optimal increase in %LS-BMD by the treatment. When the patients were divided into two groups by sCa of 9.3 mg/ml, the %LS-BMD of the every 6 month-treatment was significantly different between these two groups (0.8% vs 1.8%, $P=0.038$). Neither hypercalcemia nor hypercalcium urea were observed during 2y of the treatment of the patients in both Group 1 and 2. Same results were observed in Study 2.
Conclusion
The present study revealed for the first time that, even though within the reference range of sCa, the upper two third of sCa level is required to optimal increase in LS-BMD by BP with aVD in patients with postmenopausal osteoporosis.

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P401

Two-fold regional variation in initiation of anti-osteoporosis medication after hip fracture in the UK

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Objective
Describe UK regional variation in prescription of anti-osteoporosis drug therapy before and after a primary hip fracture during 1999–2013.
Materials and Methods
We used primary care data (Clinical Practice Research Datalink) to identify patients with a hip fracture and primary-care prescriptions of any anti-osteoporosis drugs (bisphosphonates, strontium, denosumab, oestrogen therapy, SERMS, teriparatide) prior to primary hip fracture and up to 5 years after. Regional variations in prescribing before and after generic oral bisphosphonates were analysed. Multivariable logistic regression models were adjusted for gender, age and indices of deprivation.
Results
13,069 patients (mean age 82 years, 76% female) diagnosed with a primary hip fracture during 1999–2013 were identified. Eleven percent had any prescription in the 6 months prior to primary hip fracture with no significant regional variation. In the 0–4 months following a hip fracture 5% of patients were prescribed an anti-osteoporosis drug in 1999, which increased to 51% in 2011 and decreased to 39% in 2013. Fifteen percent of patients remained on treatment by 60 months.
Independent predictors of treatment initiation included men (OR = 0.42 95% CI: 0.36–0.49), increasing BMI (OR = 0.98 95% CI: 0.97–1.00) and region (OR = 1.28 95% CI: 0.88–1.87 North East vs OR = 0.57 95% CI: 0.77–1.14 South West). Regional differences in prescribing persisted over the 5-year follow-up. If all patients were treated at the rate of the highest performing region, then nationally 3214 additional hip fracture patients would be initiated on therapy every year.
Conclusion
Significant regional differences exist in post-hip fracture prescribing of anti-osteoporosis drugs despite adjustment for potential confounders at the patient level. While a significant increase was observed after 2005, the rate of treatment initiation was still low. Further work examining differences in health care provision may inform strategies to improve in secondary fracture prevention after hip fracture.

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P402

Is recovery of lactation-induced bone loss influenced by immobilization in mice?

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Lactation in both humans and mice is associated with a substantial bone loss, in mice this bone loss is recovered within 4 weeks after weaning. This recovery is considered to be the most rapid physiological bone formation in adult life. The aim of the study was to investigate the effect of the post-lactational anabolic response on a disuse bone loss induced by Botulinum toxin (BTX).
Forty-eight NMRI mice were divided into the following groups: *pregnant*, *lactation*, *recovery + saline, recovery + BTX,* and a *virgin control* group. The lactation period was 12 days with a subsequent recovery period of 21 days. On day 1 of recovery, 2 IU of BTX/100 g body weight was injected into the right hind limb. The experiment was approved by the Danish Animal Experiments Inspectorate.
The lactation resulted in a substantial loss of bone strength (femoral neck: –63%, $P<0.001$ and femoral mid-diaphysis: –37%, $P<0.001$ vs *pregnant*) as well as a substantial loss of bone density (BV/TV: –47%, $P<0.001$, vBMD: –45%, $P<0.001$, Tb.Th: –24% $P<0.001$ vs *pregnant*). In the saline injected animals a close to complete recovery was observed, at the end of the recovery period. In the BTX injected recovery animals no additional decrease in bone strength or Tb.Th was observed compared to *lactation.* However, a decrease in bone density was observed (BV/TV: –49%, $P<0.001$, vBMD: –53%, $P<0.001$, vs *lactation*), but, interestingly, bone formation was increased (BFR/BS: 46% $P=0.002$ vs *virgin control*).
The post-lactational anabolic response prevented an immobilization induced loss of bone strength and Tb.Th, but did not mitigate the loss of BV/TV and vBMD, although an increased bone formation rate was found in BTX injected recovery animals.

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P403

Biochemical monitoring of teriparatide efficacy in a real world setting

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Teriparatide is an anabolic agent given to reduce fracture risk in osteoporosis; it increases BMD and bone turnover. For monitoring treatment efficacy, serum PINP shows the greatest increase and low variability; it has been proposed as a marker of individual treatment response. We aimed to evaluate the utility of PINP to monitor teriparatide treatment in clinical practice.
We performed a retrospective evaluation of 91 patients treated with teriparatide since PINP (Elecsys) was introduced in our centre in 2009 (81 women, mean age 71 (48–92) years, mean 5 vertebral fractures (0–13)). Lumbar spine (LS) BMD was considered uninterpretable in 47 patients due to artefacts in the region of interest (vertebral fractures, degenerative change). At baseline, mean ± S.D. BMD

T-scores at LS (reliable scans only) and total hip (TH) were -3.0 ± 1.0 and -2.3 ± 1.2 respectively; mean PINP was 35 ± 29 μg/l (5–161). Treatment response was defined as either an increase in PINP greater than the least significant change (LSC, >10 μg/l), or an increase above the premenopausal reference range (>80 μg/l).
At 3 months, 69/74 patients (93%) were defined as responders using the LSC approach and 49/85 (58%) using the absolute threshold. 40/74 (54%) patients fulfilled both criteria. Of 44 patients with reliable LSBMD at baseline, 16 had 24-month follow up, of whom 88% were defined as responders (BMD LSC, 4.5%). For THBMD, 49 had 24-month follow up, of whom 22% were defined as responders. There was no association ($r=-0.002$, NS) between 3-month PINP change and 24-month LSBMD change.
In summary, PINP identified a similar proportion of responders to teriparatide treatment in clinical practice as reported in clinical trials (using LSC criteria). LSBMD measurement was uninterpretable in many patients and early change in PINP did not predict change in LSBMD. We conclude that PINP has greater utility than BMD in monitoring individuals treated with teriparatide in clinical practice.

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P404

IV. zoledronic acid promotes healing of complicated stress fractures in the foot

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Delayed healing of stress fractures constitutes a significant clinical problem causing prolonged pain and disability for the patients affected. All stress fractures exhibit Bone Marrow (edema) Lesions (BMLs) with and without fracture lines on MRI, and in cases of delayed union these lesions persist. Previous studies on transient osteoporosis and osteoarthritis have demonstrated that bisphosphonates can reduce BMLs. We therefore wanted to test, whether treatment with iv. zoledronic acid (5 mg) given twice with an interval of 3 months causing reduction of BMLs, could promote healing in such patients.
Seven female and two male patients (aged 30–72) with non-union of stress fractures for more than 12 months were enrolled. Pain was monitored using VAS (1–10) and MRI was performed in six of the patients at baseline, 3, 6 and 12 months after the first infusion. All patients received Ca (0.5–1 g Ca per day) and vitamin D supplementation (800–1000 IE per day).
All patients experienced clinical healing with significant reduction of pain at the fracture site and improvement of ambulation within 1–3 months after the first infusion. Four patients experienced further alleviation of pain after the second infusion. At 6 months, ambulatory functions were completely restored in all patients and a highly significant reduction of VAS scores from an average of 7.3 before treatment to 1.1 at 6 months and 0.9 at 12 months ($P<0.0001$) was registered. The alleviation of pain was accompanied by partial or total resolution of the BML on MRI. Except for two cases of flu-like symptoms after the first infusion, no adverse effects were recorded.
In conclusion, treatment with iv zoledronic acid, represents a safe and effective treatment of delayed union of stress fractures in the foot, thus avoiding surgical intervention. This small observational study needs to be corroborated in a larger randomized, controlled trial.

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P405

Lithium chloride enhances bone formation and implant osseointegration in osteoporotic condition

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Osteoporotic patients have poor bone quality and high risk of dental and orthopaedic implant failure. Lithium chloride (LICl) has been reported to enhance osteogenesis. However, the role of LiCl in bone formation and implant osseointegration in osteoporotic condition is still unknown. Therefore, we aimed to investigate the effect of LiCl on bone formation and implant osseointegration in osteoporotic rats.
Three months old Sprague–Dawley female rats ($n=27$) were randomly divided into two groups and performed ovariectomy or sham surgery. After 3 months of surgery, titanium implants were implanted in tibial medullary canal. After implantation, ovariectomized rats were treated with 150 mg/kg per 2 days of LiCl (ovariectomy + LiCl) or saline, and sham-operated rats with saline by oral gavage for 3 months. Tibias with implants were harvested. Bone formation and implant osseointegration was analyzed by using histology, micro-CT, biomechanical testing, and immunohistochemistry.
Histological images clearly showed decreased number and distribution of trabeculae in tibia of ovariectomized rats. Interestingly, ovariectomy + LiCl increased number and distribution of trabeculae in tibia. Micro-CT data showed that in ovariectomy reduced bone volume tissue volume ratio (BV/TV) by 3.6-fold, trabecular number (Tb.N) by 1.8-fold, trabecular thickness (Tb.Th) by 1.6-fold, and increased trabecular space (Tb.Sp) by 2.2-fold. Ovariectomy + LiCl increased BV/TV by 1.9-fold, Tb.N by 1.4-fold, Tb.Th by 1.3-fold, and decreased Tb.Sp by 1.2-fold. Bone histology and biomechanical testing showed that ovariectomy reduced bone implant contact (BIC) by 5.2-fold, implant osseointegration by 5.4-fold, and implant mechanical fixation by 5.5-fold. Ovariectomy + LiCl increased BIC by 4.4-fold, implant osseointegration by 3.3-fold, and implant mechanical fixation by 2.9-fold. Ovariectomy + LiCl increased β-catenin expression by bone cells around the implants.
In conclusion, our study shows that LiCl enhances bone formation and implant osseointegration in osteoporotic rats, suggesting LiCl as a promising therapeutic agent to increase bone mass as well as to prevent implant failure in osteoporotic condition.

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P406

Adherence and persistence to teriparatide treatment in patients attending a specialised Bone Health Service

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Introduction
Osteoporosis affects the bones causing weak or brittle bones and increasing the risk of fracture. Efficacy of anti-osteoporotic treatment is based on drug potency and adherence and persistence. Teriparatide (TPTD) is the first anabolic agent developed for the treatment of osteoporosis. It is usually given as a daily subcutaneous injection for a two-year treatment course.
Aim
To evaluate adherence and persistence to TPTD treatment in patients, affected by severe osteoporosis in a Specialised Bone Health Service.
Method
A cross-sectional and retrospective longitudinal study of 473 patients with severe osteoporosis treated with TPTD was carried out between 2004 and 2015.
Results
473 patients commenced TPTD between 2004 and 2015. Females 96%. Mean age 71 years, range 30–99 years. 301 (64%) had previous fractures, 177 (37%) had vertebral fractures. 263 (56%) completed the course, 125 (26%) still on treatment, 86 (18%) prematurely stopped. For the latter the mean length of time on treatment was 195 days, median 147 days, range 2–609. Reasons for prematurely stopping include Death 6 (7%), self-discontinued 14 (16%), cancer 4 (5%), lost to follow up 4 (5%),side effects 57 (67%). Some patients experienced multiple side effects which included rash 2(3%), Dizziness 7 (12%), Nausea 11(19%), Headaches 4(7%), Fatigue 7 (12%) and Generalised/Joint pain 29 (51%).

Discontinuation by age group

Age group	Number on treatment	Number discontinued
30–49 years	13	1 (8%)
50–69 years	189	31 (16%)
70–89 years	261	39 (15%)
90–99 years	8	2 (25%)

Conclusions
These results show that adherence and persistence with TPTD is higher than with oral antiresorptive treatments.The major factor that reduced adherence and persistence was tolerability. Interestingly age did not appear to be an important

factor in premature discontinuation of the treatment. These findings are important as numerous studies have shown that high adherence and persistence with antiresorptive therapies are necessary to ensure an optimal therapeutic outcome.

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P407

Impact of 3-year vitamin D and calcium supplementation on mineral and organic matrix formation of trabecular bone in postmenopausal osteoporosis

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Clinical trials involving drug therapies for postmenopausal osteoporosis typically compare effects of the active drug combined with vitamin D (vit D) and calcium (Ca) vs vit D and Ca supplementation on its own. Bone strength is estimated based on the amount of bone, frequently expressed as bone mineral density determined by dual X-ray absorptiometry, and quality of bone, hardly measured in clinical practice.

The purpose of the present study was to compare bone material composition properties at actively bone forming (based on fluorescent labels) trabecular surfaces between two independent, age-matched groups: treatment-naïve postmenopausal osteoporosis patients with various level of vit D and Ca supplementation according to the routine clinical practice (TN; controlled vit D and Ca supplementation for up to 3 months) and patients who participated in the placebo arm of the prospective HORIZON clinical trial for zoledronic acid and received adequate vit D and Ca supplementation for 3 years (PLC). We analyzed by Raman microspectroscopy iliac crest biopsy samples by ANOVA for: the mineral/matrix ratio (MM); nanoporosity (a surrogate for tissue water content); the mineral maturity/crystallinity (MMC); the glycosaminoglycan (GAG) content; and the pyridinoline (Pyd) content. The results indicate significant differences between the two groups in all monitored parameters even after adjustment for tissue age. Specifically, the PLC group exhibited lower MM (-53%, $P<0.0001$) and GAGs (-90%, $P<0.0001$), and higher nanoporosity ($+89\%$, $P<0.01$), MMC ($+5\%$, $P<0.001$), and Pyd ($+62\%$, $P<0.0001$) values compared to TN.

Given that major clinical trials in osteoporosis involve comparison between active drugs supplemented with adequate dose of vit D plus Ca, and adequate dose of vit D plus Ca, the difference in forming bone quality between these two groups of patients may underestimate the difference between patients on adequate osteoporosis therapies and treatment naive patients from the clinical practice.

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P408

Treat-to-target in osteoporosis. Mith or reality? Results of a Spanish Delphi study

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Objective

To define, by expert consensus, the criteria for the application of a Treat-to-Target (T2T) strategy in osteoporosis, in Spain, and to assess the adequacy of current treatments for it.

Material and methods

Six Spanish experts in osteoporosis formed the Scientific Committee that led the project and designed the questionnaire used in two Delphi rounds. The 24 items included in the questionnaire assessed the experts' wish (W) and prognosis (P) for each item to occur in 5-year time, in a seven-point Likert scale (1 = entirely disagree; 7 = entirely agree). Second round included items without consensus in the first. Consensus was defined as $\geq 75\%$ of agreement (5–7) or disagreement (1–3) responses.

Results

The first round was completed by 112 out of 165 experts and the second by 106. A total of 59.8% of participants were rheumatologists with a mean of 21.3 years (s.d.: 8.5) of clinical experience.

There was consensus on 70% of items. Consensus was established in the utility of T2T strategy to define therapeutic objectives, optimal follow-up and, therapeutic algorithm (W:96.4%; P:82.1%). Experts agreed on the utility of lack of fractures (W:99.1%; P:97.3%), bone mineral density (BMD) (W:91.1%; P:91.1%) and fracture risk reduction by FRAX (W:75.9%; P:84.0%) as therapeutic objectives. Treatment failure was defined as no BMD gain after 2 (W:81.3%; P:82.1) or 3 years (W:77.7%; P:75.9%), new fracture diagnosis within 2 (W:92.0%; P:92.0) or 3 years (W:90.2%; P:88.4%) or the absence of bone turnover markers (BTM) change after 6 months (W:75.0%; P:93.4%) or 1 year (W:90.6%; P:89.6%) of treatment. Except for strontium ranelate (W:76.4%; P:58.5%), consensus was reached for all available and upcoming novel therapies to achieve a therapeutic target trough T2T strategy application.

Conclusion

A T2T strategy in osteoporosis can be implemented in Spain, since therapeutic objectives, treatment failure and appropriate treatment choice for this strategy have been established.

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P409

Atypical femur fracture in an adolescent with X-linked osteoporosis based on PLS3 mutation

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Background

Long-term use of bisphosphonates has raised concerns about the association with Atypical Femur Fractures (AFFs) that have been reported mainly in postmenopausal women.

Clinical case

An 18-year-old patient with juvenile osteoporosis based on X-linked osteoporosis due to a PLS3 mutation developed a low trauma femoral fracture after seven years of intravenous and two years of oral bisphosphonate use, fulfilling the revised ASBMR diagnostic criteria of an AFF. The occurrence of AFFs has not been described previously in children or adolescents. The underlying monogenetic bone disease in our case strengthens the possibility of a genetic predisposition of these fractures. We cannot exclude that a transverse fracture of the tibia that also occurred after a minor trauma at age 16, followed by a spontaneous fracture above the intramedullary tibial nail, might be part of the same spectrum of atypical fractures related to the use of bisphosphonates. In retrospect our patient experienced prodromal pain prior to both the tibia and the femur fracture. Case reports of atypical fractures in children with a monogenetic bone disease such as osteogenesis imperfecta (OI) or juvenile osteoporosis are important to consider in the discussion about optimal duration of bisphosphonate therapy in growing children.

Conclusion

This case report i) highlights that AFFs also occur in adolescents treated with bisphosphonates during childhood and pain in weight-baring bones can point towards this diagnosis ii) supports other reports suggesting that low trauma fractures of other long bones besides the femur may be related to long-term use of bisphosphonates iii) strengthens the concept of an underlying genetic predisposition of AFFs, now for the first time reported in X-linked osteoporosis due to a mutation in PLS3 and iv) should be considered in decisions about the duration of bisphosphonate therapy in children with congenital bone disorders.

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P410

Can bone turnover markers help to define the duration of bisphosphonate drug holidays?

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Background
On cessation of bisphosphonate treatment, while bone mineral density decreases slowly, bone resorptive markers such as C-terminal telopeptide (CTX) increase more quickly and may be useful in monitoring 'offset' of action. Our aim was to analyse changes in CTX on stopping long-term bisphosphonate treatment to guide clinical decision-making on the duration of treatment cessation ('drug holidays').
Subjects and methods
A total of 158 patients (83% female, mean age 71 years) starting a *drug holiday* had plasma CTX measured at baseline ($n=138$), 4 months ($n=136$) and 12 months ($n=100$). *Offset* of action was defined as a rise by the least significant change (LSC = 33%) in CTX or CTX above the pre-menopausal mean (0.19 ug/l).
Results
Mean (SD) duration of therapy was 8 (2.7) years, with 59% stopping alendronate and 33% risedronate. At baseline, 32% of CTX measurements were above the pre-menopausal mean, while mean [median, IQR] CTX was 0.18 [0.16, IQR 0.11-0.22] ug/l rising at 4 months to 0.21 [0.20, 0.14–0.26] ug/l and 12 months 0.24 [0.23, IQR 0.18–0.29] ug/l. At 4 months, 47% patients showed a rise in CTX above LSC. At 12 months, 69% were above LSC and 66% were above pre-menopausal mean. No detectable changes in CTX were seen in 31% of patients over 12 months.
Conclusion
We found that, after at least 5 years of treatment, CTX may not be adequately suppressed in a third of patients for whom drug adherence should be reviewed. Treatment effects can wear off as quickly as four months but may also be maintained up to a year. However, monitoring of CTX can identify these patients, some of whom may need to restart treatment earlier.

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P411

Establishment of a large sector-spanning fracture liaison service in Germany

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Patients with osteoporotic fractures are frequently treated in trauma surgery. While fracture fixation is at the center of patient care, treatment of the underlying bone disorder is often not considered, thereby increasing the risk for subsequent fractures. Closing this treatment gap is therefore among the greatest challenges in modern trauma surgery. To address this problem, we established a fully structured, multidisciplinary, sector-spanning fracture liaison service (FLS), which is among the first and largest in Germany serving a population of about two million people. Residents in trauma surgery at the local University Hospital who are dedicated to the FLS identified inpatients with an incident fragility fracture, focusing on postmenopausal women and men over 60 years of age. Next, patients answered a questionnaire to assess risk factors for fragility fractures and were provided with comprehensive information about the disease and the possibility to be treated within the FLS. All patients were free to choose one of 20 osteoporosis experts participating in the network to be referred to for further diagnosis and treatment. The multidisciplinary FLS consists of specialists in trauma- and orthopedic surgery, endocrinology and internal medicine, all working in private practice. Preliminary results demonstrate that about 20 patients per week can be included in the FLS, an efficacy that reaches more than 90% of patients fulfilling the inclusion criteria. To reach this goal, a highly motivated clinical FLS team is essential. Difficulties were encountered with patients suffering from dementia or other conditions compromising compliance. More clinical and logistic data are currently acquired and evaluated. We conclude that a multidisciplinary FLS is a powerful interface between inpatient surgical care and outpatient osteoporosis treatment. This system may reach the majority of patients with fragility fractures and can subject them to an adequate treatment, which will reduce morbidity, mortality and the socio-economic costs.

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P412

The value of the central vertebral height restoration by kyphoplasty for treatment of biconcave vertebral compression fractures

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Purpose
To study the value of the central vertebral height restoration by kyphoplasty for treatment of biconcave vertebral compression fractures.
Method
Review the patients with the single biconcave vertebral compression fracture receiving kyphoplasty treatment from 2006 to 2013 (49 cases, 13 males and 36 females) According to Genant classification, with the central vertebral compression ratio, they are divided into type II (<25%, 11 cases), type II (25–40%, 26 cases), type III (> 40%, 12 cases). The expected recovery height is the average of the anterior and posterior edge. According to the ratio of achieved height/expected recovery height, it is divided into level A (60∼80%), level B (60∼80%), level C (<60%). Type I patients are mild deformity pre-operation and level IA (11 cases); type II patients are moderate deformity pre-operation and level IIA (12 cases) and level B(14 cases) post-operation, type III patients are sever deformity pre-operation and level IIIA (2cases) and level IIIB (3 cases) and IIIC (7 cases) post-operation. Bone cement leakage are followings: type I 1 case (1/11); type II 11 cases (11/26) type III 6 cases (6/12). Patients were followed up 24-84 months. Analyze the VAS and ADL score of each group of pre-operation, 1 day, 3 months, 1 year, 2 year follow-up.
Result
The scores of VAS and ADL were significantly better than pre-operation, and there was no significant difference between 2 years and 3months follow-up. When it comes to bone cement leakage, with the increase of the degree of fracture, the incidence of bone cement leakage is gradually increasing. The bone cement leakage rates of type II and III were significantly higher than type I.
Conclusion
Both short and long term effect of kyphoplasty for the treatment of biconcave compression fractures is preferable. For moderate and severe compression fractures, it has no significant effect whether the central vertebral height has been recovered, but the bone cement leakage rate was significantly increased. Therefore, we should not insist restoration of the central height when treating biconcave compression fractures.

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P413

Dose postoperative applications of diphosphonate medicine at an early stage have influence on union of fracture?

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Background
Surgical and conservative treatments are both crucial method to deal with osteoporotic fracture, anti-osteoporotic therapy is the most important among conservative treatments and diphosphonate medicine is the first-line choice. The pharmacological action of diphosphonate medicine is to inhibit the function of osteoclast, so there is no consensus if it will inhibit porosis and union of fracture.
Purpose
To find out the influence of diphosphonate medicine to osteoporotic distal radial fracture retrospectively.
Method
From February of 2011 to March of 2014, total 78 patients suffered from distal radial fracture whose age is 57.3 ± 15.8 including 31 male patients and 47 female patients. Open reduction and internal fixation have been performed to all patients; the type of fracture and postoperative bone density both have been recorded. 26 cases used zoledronic acid at 3 days after operation are divided into group A, 16 cases used zoledronic acid at 3 months after operation are divided into group B, 24 cases used alendronate at 3 months after operation are divided into group C, and 10 cases used nothing are divided into group D. We evaluated the union of fracture according to clinical test and radiological data at 3 months and 6 months after operation, and we retest the bone density at 6 months after operation. We chose SPSS17.0 to compare the rate of union and bone density among these groups.
Result
Preoperative type of fracture and bone density have no significant difference among each group. As to union of fracture, the rates of union at 3months after operation are 21/26 (group A), 13/18 (group B), 19/24 (group C), 9/10 (group D), no significant difference has been found. The rates of union at 6 months after

operation are 24/26 (group A), 18/18 (group B), 23/24 (group C), 10/10 (group D), no significant difference have been found either. The bone density at 6 months after operation of group A is higher than group D, but no difference has been found.

Conclusion

Applications of diphosphonate medicine at an early stage for osteoporotic distal radial fracture has no significant influence to the union of fracture. Bone density has no significant influence at the early stage either and further observation should be taken.

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P414

Teriparatide reduces the risk of vertebral fractures compared with standard care in patients with severe osteoporosis

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Objectives

Treatment outcomes in patients treated with teriparatide were compared to standard care in patients with severe osteoporosis (bone mineral density *T* score of −4 or less).

Methods

An observational study was performed of patients attending a specialist osteoporosis service and who were initiated on teriparatide ($n=324$). Patients that met criteria for teriparatide but declined treatment, were already established on a bisphosphonate or had a contraindication, formed the control group ($n=148$). An intention to treat analysis was performed including 35 patients that discontinued teriparatide treatment due to side effects, difficulty with treatment or death. The frequency of fracture after 3 years of follow-up were compared using χ^2 test.

Results

Over the observation period patients receiving teriparatide were significantly less likely to suffer a vertebral fracture, though rates of non-vertebral fracture did not differ between the groups. Patients that received treatment with PTH were older and more likely to be female, though no difference in BMI was observed (Table 1).

Table 1 Characteristics of patients treated with teriparatide (PTH) or controls.

	Age	Female sex	BMI	New Vert #	New Non Vert #
Control	73±9.1	130 (88%)	23.37	9 (6%)	16 (10%)
PTH cohort	70±9.8	305 (94%)	23.24	7 (2%)	31 (10%)
P	<0.05	<0.01	ns	<0.05	ns

Conclusion

In patients with severe osteoporosis, and particularly those at risk of vertebral fracture, teriparatide may be the preferred treatment option.

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P415

rs72658163, a new heterozygous variant in COL1A2 associated with atypical femoral fracture

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Atypical femoral fractures (AFF) of the subtrochanteric region are rare. Bisphosphonates account to a large extent to their occurrence, however AFF also occur without exposure to bone medication. We here assessed the genetic factors associated with AFF among subtrochanteric fractures. Cases of subtrochanteric or femoral shaft fractures were identified through ICD-10 codes in three French academic centers from 2007 to 2010. Medical records were analyzed by two investigators that adjudicated X-rays for typical or atypical fractures. Among them, genetic screening for ALPL or COLA1/A2 variants was performed after patient's information and consent. A total of 389 cases were identified and 268 were ruled out. On the remaining 121, 14 (11.6%) patients had AFF. No clinical characteristic differed between groups. In the AFF group, four were exposed to bisphosphonates and one to raloxifene. Genetic analysis was could be performed in five patients and found one patient with a heterozygous mutation in COL1A2 gene (NM_000089.3:c.2123G, rs72658163, p.Arg708Gln) and three patients with various heterozygous ALPL mutations of unknown significance. This mutation in COL1A2 has been previously described in a patient with atypical Marfan syndrome. Our patient was 78-year-old and did not show any sign of Marfan syndrome. She had been treated more than 5 years by risedronate. *In sillico* analyses were performed showing that this variant is found in less than 0.1% of the population and is predicted to be probably damaging with a score of 1.000 and previous studies on this variant show that it does impact collagen fibril assembly and may therefore have a role as a modifier in disease pathogenesis. In conclusion, although the use of bisphosphonates is a major contributor to subtrochanteric femoral fractures. Genetic variants in COL1A2 genes was found in one out of five patients and could be a genetic background involved in this event.

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Hydrogen sulfide is a novel regulator of bone formation involved in the bone loss induced by estrogen deficiency

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Hydrogen sulfide (H_2S) is a gaseous molecule produced endogenously in mammalian cells. H_2S was recently found to play important roles in the regulation of inflammation, redox homeostasis and cell lifespan. Moreover, H_2S was shown to maintain mesenchymal stem cell (MSC) function and stimulate osteogenic differentiation of MSC. However, it is unclear whether H_2S plays any role in the bone loss induced by estrogen deficiency.

The objective of this study was to investigate the role of the endogenous pathway leading to H_2S generation in ovariectomy (ovx)-induced bone loss in mice.

We found that ovx induced a 29% decrease in femural BV/TV relative to sham mice ($P<0.0001$). Moreover, ovx decreased serum H_2S levels by 65% ($P<0.001$) and blunted the bone marrow (BM) mRNA expression of the two key H_2S-generating enzymes ($P<0.001$), cystathione β-synthase (CBS) and cystathione γ-lyase (CSE). To restore H_2S levels, mice were treated with a common H_2S-donor drug, GYY4137 (GYY) (1 mg/mouse, every other day) for 4 weeks and indexes of bone mass were measured by μCT and hystomorphometry. We found that treatment with GYY normalizes serum H_2S levels in ovx mice, and completely prevents the loss of femural BV/TV. Hystomorphometry revealed that GYY induced a significant increase in N.Ob-BS and Ob.S/BS ($P<0.05$). Furthermore, GYY-treated mice significantly increased serum levels of P1NP, a marker of bone formation, and increased CFU-ALP formation ex vivo by 28% relative to ovx mice ($P<0.0001$). Treatment with GYY resulted in increased WNT signaling in BMSCs and increased the expression of the WNT ligands Wnt16, Wnt2b, Wnt6 and Wnt10b in the BM. Experiments performed in human BM cells confirmed that H_2S stimulates osteogenic differentiation of MSC. In summary, we show that ovx is associated with impaired H_2S synthesis in mice, and that pharmacological replacement of H_2S stimulates bone formation preventing the bone loss induced by estrogen deficiency.

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The fate of injected cement after percutaneous vertebroplasty

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Introduction
Percutaneous vertebroplasty is a well known surgical method to put the bone cement at osteoporotic vertebrae. There was a few report concerning about bone cement related complication such as dislodgment of injected cement. There are some contradictory study for long term reaction to bone cement in osteoporotic bone, especially injected cement features. This study is to assess the radiographic features 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 radiologic outcome focused on injected cement feature.
Results
The mean follow-up period was 8.2 years. A new adjacent vertebral fracture was documented by 55 vertebral bodies in 35 patients. 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.
Discussions
Most of the injected cement showed radiologically stable in cement-bone interface, anterior vertebral height or focal kyphotic angle. In order to get more strengthened vertebrae, more biocompatible material will be needed.

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Globular adiponectin reverses trabecular osteopenia in ovariectomized rats

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Adiponectin has recently been shown to influence skeletal metabolism. Over-expression of adiponectin gene suppresses bone resorption and increases trabecular bone mass. Bone marrow stromal cells from adiponectin-*null* mice had reduced mineralized nodule formation ability compared with wild type mice. Adiponectin, signalling via the adiponectin receptor 1 (AdipoR1) stimulates osteoblast differentiation and inhibits osteoclastogenesis. We have shown that the globular form of adiponectin (gAd) signals via AdipoR1 and stimulates osteoblast differentiation. Based on this observation, we hypothesized that gAd could restore bones in osteopenic rats.
Methodology
A prior approval from Institutional Animal Ethics Committee was obtained. Endotoxin free recombinant gAd was prepared in-house. Adult Sprague dawley rats (200±10 g) were either sham-operated (ovary intact) or ovariectomized (OVX) and left untreated for 12 weeks for trabecular osteopenia to develop in the OVX group. Rats were then randomized into following groups ($n=8$); sham+vehicle (0.9% saline); OVX+vehicle, OVX+gAd (10 μg/rat, i.p.), OVX+gAd (100 μg/rat, i.p.), OVX+hPTH (1–34) (40 μg/kg/day, 5 days/week, i.p.) and OVX+alendronate (3 mg/kg/day, orally). Bone parameters were measured by micro-computed tomography. Data are expressed as mean±S.E.M., data analysed using non parametric ANOVA followed by Dunnett's multiple comparisons test using GraphPad Prism5.
Results
OVX+vehicle group showed a dramatic loss of trabecular bones at distal femur compared with the sham (control). gAd had a dose-dependent effect in restoring the trabecular bones of OVX rats. At its highest dose (100 μg), gAd restored trabecular bone volume per tissue volume (BV/TV%), trabecular thickness, trabecular number and trabecular spacing to the level of control. The skeletal effect of PTH however, was superior to gAd at its highest dose. (Table 1)

Table 1.

	Sham	Ovx+Veh	Ovx+PTH	Ovx+ALN	Ovx+ gAd10	Ovx+gAd100
BV/TV	20.2±1.2***	10.6±1.3	24.4±1.4***	16.2±0.6**	16.0±1.5**	17.4±0.5***

$P>0.05$*, $P\leq0.05$, **$P\leq0.01$, *** $P\leq0.001$.

Summary
In a preclinical model of post-menopausal osteoporosis, we showed therapeutic efficacy of gAd in restoring trabecular bones.

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Other diseases of bone and mineral metabolism

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Natural history and prognostic factors of fibrous dysplasia of bone in a modern cohort of 372 patients. The Francedys Study

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Fibrous dysplasia of bone (FD) is a rare inherited but sporadic bone disease that can be responsible for bone pain, fracture and bone deformity. The prognosis may be difficult to establish because of the wide spectrum of disease severity, with patients benign forms of the disease and some others who are severely affected. We have analyzed the data from the French National reference center for FD. We have established a database from electronic medical records. We have made descriptive statistics of the various forms of FD and examined the prognostic factors by multivariable logistic regression analysis, with a parcimonious stepwise method. The primary outcome was a clinically relevant composite index combining bone pain (analogic scale >3) and/or incident fracture.
In our modern cohort of 372 patients, the median age at diagnosis was 23. The revealing symptom (median age=18) was bone pain in 44% of patients, a fracture in 9% but the diagnosis was fortuitous in 25% of cases. Monostotic forms represented 58% of patients and polyostotic forms 42%. The femur was the most commonly affected bone (44% of patients), followed by the skull (38%). Twelve percent of patients had McCune-Albright syndrome (MAS). With a median duration of follow-up of 7 years among 211 patients, we observed an incidence of fracture of 17 and 51% of patients had no bone pain at the end of follow-up. In univariate analysis, younger age at diagnosis, renal phosphate wasting, a polyostotic form and prevalent fracture were predictors of a poorer prognosis, but in the multivariate model, the polyostotic form was the only significant predictor (OR=2.04 [1.29–3.27]).
In conclusion, in a national referral center for FD, one patient on follow-up out of six had incident fracture. A polyostotic form was the main risk factor of a poorer outcome.

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Calcification in the vessel wall: impact of vitamin K dependent proteins

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Pathophysiological calcification in the vasculature favours cardio- and cerebrovascular diseases. In patients with chronic kidney disease vitamin K metabolites are associated with decreased vascular calcification.

We investigated the expression of vitamin K dependent proteins (VKDPs) in vessels and bone to identify differences in expression pattern during atherosclerosis (AS) stages and compare the two tissue profiles.
Gene expression levels of BSP, MGP, OC, TGFBI, GRP, GAS6, periostin, PRRG1-4, protein Z and S were examined with predesigned TaqMan assays on a LC480 system in vessels (external iliac artery and aorta) and bone of 26 organ donors. Relative Cp values were obtained by division with beta actin.
Determination of calcification stages was done histologically: no changes: unaffected vessels, intima thickening: more than one-fold intima thickening without calcification, intima calcification: one or more calcification spots.
Gene expression of MGP, TGFBI, GAS6, periostin, protein S and PRRG1 decreased in bone compared to vessels in atherosclerosis ($P=0.001$, $P<0.001$, $P<0.001$, $P<0.001$, $P=0.001$, and $P=0.001$).
In vessels gene expression of GRP, PRRG1, 3 and 4 were decreased in atherosclerosis compared to normal state ($P=0.037$, $P=0.002$, $P=0.011$, $P=0.011$). In bone gene expression of BSP decreased when atherosclerotic vessels were present ($P=0.018$).
Looking at three stages of atherosclerosis, differences in gene expression of TGFBI ($P=0.023$) and periostin ($P=0.002$) are seen in intima thickening, in intima calcification also MGP ($P=0.007$), GAS6 ($P<0.001$), protein S ($P=0.002$) and PRRG1 ($P=0.001$) show differences in gene expression in bone and vessels.
In vessels PRRG1 ($P=0.013$), 3 ($P=0.048$) and 4 ($P=0.049$) gene expression decreased during intima thickening and keeps low in the calcification stage. In bone gene expression of VKDPs did not change during AS progression.
Gene expression of vitamin K dependent proteins changes during calcification of the vessel wall, implicating a more complex role of vitamin K dependent proteins in vascular calcification than previously known.

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Postmenopausal osteoporosis and periodontal disease about 60 cases

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Introduction
Menopause plays an important role in the onset and/or worsening of periodontal disease by the action of sex hormones on the various body systems. Several studies have reported an association between osteoporosis and periodontal destruction. The aim of this study was to evaluate the effect of postmenopausal osteoporosis on periodontal status.
Material and methods
This is a case-control type study, conducted among 30 women attending the Rheumatology Service of the University Hospital Ibn Roch of Casablanca for postmenopausal osteoporosis and 30 postmenopausal women without osteoporosis recruited in the various services of Surgical Dentistry, Prosthetic Joint Consultations and Dental Treatment Facility (CDPF) in Casablanca. The inclusion criteria were; postmenopausal osteoporosis in cases and menopause in control group along with a reason other than periodontal disease. Women should have at least seven teeth. The exclusion criteria were the general diseases affecting oral health, patients on general medications, those who smoked and those who have had a hysterectomy. Periodontal variables were assessed clinically (level of plaque, degree of gingival inflammation, probing depth, attachment loss, mobility, recession and lysis inter-radicular). The DMF index is a composite indicator of carious damage that counts the number of teeth decayed, missing or blocked. Statistical analysis of the data was made by the EPI-INFO 6.0 software Fr. The test used for the comparison of numbers was the χ^2-test. The test used for the comparison of means was Student's test.
Results
The majority of patients were aged between 51 and 60 years (53.4% for cases and 60.1% for the control group) with an average of 57.93 ± 7.14 in osteoporotic patients, and 54.26 ± 6.85 in controls. The mean follow-up of osteoporosis was 3 years old. In this group, all patients were on oral bisphosphonates. No patient received prior hormone replacement therapy for menopause. In both groups, all women had a low socioeconomic level. The average Body Mass Index was 27.25 ± 3.10 to $27.52 \pm$ cases and 2.44 in controls ($P=0.01$). There was no significant difference in the DMF index between osteoporotic patients and control patients (13.10 ± 6.69 and 13.40 ± 6.02) ($P>0.05$). In contrast, patients with osteoporosis had worse dental hygiene: significant plaque accumulation in 53.1% vs 16.5% ($P=0.001$) and gingival inflammation more pronounced (26.5%) compared with controls (9.9%) ($P=0.007$). Loss of significant attachment, located mainly at the incisors and lower molars was found in women with osteoporosis (40%) compared to controls (16.6%), $P=0.04$. No cases of osteonecrosis of the jaw was found.
Discussion
Several studies and clinical research have shown that postmenopausal osteoporosis is a major risk factor for periodontal disease. In osteoporotic patients, the prevalence and risk of developing periodontitis are higher. Probing depth, attachment loss and its severity increase significantly depending on the age and poor hygiene. After this study, we found that the oral hygiene, condition of the teeth and periodontal environment, present the main factors responsible for periodontitis in osteoporotic postmenopausal women.
Conclusion
In light of these results, we stress the need for health education and the establishment of a preventative oral health in osteoporotic postmenopausal women.

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Bone loss and coronary artery disease, about 46 cases

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Introduction
Osteoporosis (OP) as cardiovascular diseases are causes of morbidity and mortality. Several publications have shown a relationship between the OP and coronary heart disease (MC). Their coexistence was considered separate conditions related to age, and was mainly attributed to aging and common risk factors including diabetes, dyslipidemia, and smoking.
Study Objective: To assess the prevalence of BMD bone loss in patients treated for coronary artery disease compared to a control population.
Materials and methods
This is a prospective, case-control, bi-centric longitudinal bearing CHD patients followed for CHU Ibn Rushd Cardiology Service of Casablanca between 2014 and 2015. The exclusion criteria were all patients with pathology weakening the bone. Patients were divided into two groups, one group bearing coronary artery disease and the second control group with normal coronary angiography. All patients underwent a complete physical examination, a complete calcium and phosphate, metabolic, and bone densitometry.
Results
The study was conducted in 46 patients, including 22 men and 24 women. The mean age was 65.7 ± 6.5 years. For the record, they found 34% had diabetes type II, 60% on insulin, 21% had dyslipidemia, 80% on statins, 32% had hypertension and 17% were chronic tobacco. Of the 46 patients, 10 (21.7%) had osteoporosis, 19 (41.3%) had osteopenia, and 17 (37%) had normal bone mineral density. The prevalence of osteoporosis and osteopenia was significantly greater in group I than in group II.
Discussion
Szulc *et al*. found an association between BMD, bone turnover markers and risk of myocardial infarction and stroke in 744 men over 50 years. Even since 1990, the Framingham study showed an inverse relationship between cortical thickness of the metacarpals and the risk of occurrence of coronary events, and after adjustment for cardiovascular risk cofactors.
In this sense, numerous publications describe a link between bone fragility and cardiovascular disease, but never establish proof of a cause-effect relationship.
Conclusion
Several studies have shown the association between osteoporosis and cardiovascular disease. The mechanisms are multiple and still imperfectly understood. It seems legitimate to propose a DXA sick "vascular" and a cardiac evaluation with osteoporotic subjects.
Conclusion
In light of these results, we stress the need for health education and the establishment of a preventative oral health in osteoporotic postmenopausal women.

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Vitamin D status in patients followed for chronic liver diseases

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Introduction
Osteoporosis is the musculoskeletal disease, the most common complication of liver osteodystrophy. Combined with vitamin D deficiency, they expose the patient to increased risk of fracture, increased morbidity and impaired quality of life.
The objective of the study is to evaluate the vitamin D status and the prevalence of densitometric osteoporosis in 100 patients followed for chronic liver diseases.

Materials and methods
Prospective study conducted between October 2014 and March 2015. This was a cohort of 100 patients followed for chronic liver disease, gastroenterology department at CHU Ibn Rochd of Casablanca. Were excluded patients with other pathology can induce a debilitating osteopathy (malabsorption syndrome, …). All patients benefited from an assessment of bone mineral density by densitometry (DEXA) and a calcium and phosphate (serum calcium, urinary calcium 24 h, serum phosphorus, phosphaturia 24 h, 25-OH Vitamin D). The desired outcomes were prevalence of vitamin D deficiency and bone loss and its risk factors.
Results
These were 100 patients. The average age was 53 ± 16 years. The Sex ratio (M/F) was at 0.75. The average duration of evolution of liver disease was 29 ± 31 mois. Thirty-five percent were followed for chronic viral hepatitis C, 31% post hepatitic cirrhosis, other causes were undetermined origin type of cirrhosis, chronic viral hepatitis B, primary biliary cirrhosis and autoimmune hepatitis. The average vitamin D was 18.24 ng/ml (SD 8.54), the mean serum calcium was 2.3 mmol/m, urinary 4.7 mmol/24 h Phosphoémie to 2.61 mg/l and urine to 16.03 mmol/24 h. 39% of patients had osteoporosis and 33% osteopenia. Among the raised risk factors, there was the low body mass index, the alcoholysis and smoking.
Discussion
The hypovitamin D is common in chronic liver diseases. It is involved in high prevalence of osteoporosis densitométrique. 12–45% depending on the series regardless of the etiology. In our study, bone loss was present in more than 2/3 of patients, regardless of the etiology of liver disease: 39–33% with osteoporosis and osteopenia. The therapeutic management involves systematically general measures including a balanced diet, alcohol and tobacco cessation and regular physical activity and specific treatment with a vitamin and calcium supplementation and bisphosphonates according to precise indications.
Conclusion
The vitamin D deficiency and bone densitometry loss are common in patients with chronic liver disease, cholestatic or not. Most often multifactorial, the vitamin and calcium assessment and ostéodensitométric must be systematic in all chronic liver disease monitoring balance sheet to prevent the occurrence of fractures, source of morbidity and mortality.

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Bone mineral density changes in kidney transplant recipients

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Bone mineral density (BMD) changes were estimated in kidney recipients. In 63 patients aged 24–64 years, measured creatinine clearance at the end of the first posttransplant year was > 50 ml/min and remained stable over the study period, bone mineral density (BMD) was estimated in the lumbar spine, femoral neck, Ward's triangle and the distal third of the radius using dual energy absorptiometry (DEXA) 0.1–4, 12–18 and 23–36 months after transplantation. IPTH, Ca and Pi were measured 1–8, 12–18 and 23–36 months posttransplant. BMD changes (delta BMD, ±%) between measurements were calculated per 12 months. Data are given as median, lower and upper quartile. Delta BMD (±%/12 months): i) first: second measurement; lumbar spine −3.02 (−5.91, 0.001), femoral neck −1.94 (−6.66, 3.70), Ward's triangle −3.9 (−2.5, −0.9), distal radius −1.3 (−3.34, 0.16), ii) second: third measurement; lumbar spine 1.76 (−0.96, 4.24), femoral neck 1.82 (−0.56, 5.13), Ward's triangle 0.495 (−4.86, 6.95), distal radius −0.87 (−2.65, 1.13). Delta BMD for radius did not differ significantly between two observational periods. Dialysis duration prior to transplantation correlated significantly negatively with radius T and Z scores at all measurements. PTH correlated significantly negatively with femoral neck BMD at first measurement. IPTH was higher in patients who lost radial BMD between the first and second measurement than in those who did not. IPTH values did not differ significantly between measurements. During the first posttransplant year, the majority of patients lost cortical and trabecular bone. For cortical bone, this trend continued after this period. Dialysis vintage prior to transplantation was a risk factor for cortical bone loss. Hyperparathyroidism influenced bone negatively.

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IL-6/STAT3 pathway is critically involved in vascular calcification via histone modification of the RUNX2 promoter in vascular smooth muscle cells

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Objective
Both inflammation and vascular calcification have been known as independent risk factors of cardiovascular events. However, the induction mechanism of vascular calcification by inflammation is still unclear. We here assessed the molecular effects of pro-inflammatory cytokines on vascular smooth muscle cell (VSMC) calcification.
Methods
VSMCs from human aorta cultured in osteoblast induction medium (OIM) and stimulated by pro-inflammatory cytokines, such as IL-6/sIL-6R (100 ng/ml), TNF-α (10 ng/ml) and IL-1β (2 ng/ml). Expression of mRNA and protein was determined by quantitative real-time PCR and WB, respectively. Cell calcification was evaluated by Alizarin Red S staining on day 21. Histone modification of RUNX2 promoter was determined by ChIP-PCR.
Results
Among pro-inflammatory cytokines, IL-6 caused the greatest induction in calcification of VSMCs. Stimulation with IL-6 for 3 days significantly increased mRNA expression of RUNX2, a master regulator for osteoblast differentiation, and ALP (3–5 and eightfold vs control, respectively) but decreased mRNA expression of SM-MHC (0.2-fold vs control), a SMC differentiation marker. IL-6 also increased phosphorylation of STAT3 and STAT3-binding to the Runx2 promoter region in VSMCs. STAT3-knockdown of VSMC with siRNA inhibited both IL-6-induced calcification and RUNX2 expression. To elucidate the relationship between histone modification and STAT3-dependent transcription of RUNX2, we next examined the level of histone modification on the Runx2 promoter region after stimulation with or without IL-6 for 20 min. The level of H3K9ac, H3K14ac, H3K4me3 and H3K36me3 was similar in both groups, while the level of H3K9me3, a repressive mark, was strongly decreased in VSMCs treated with IL-6. In addition, IL-6-dependent H3K9me3 suppression was canceled by STAT3 siRNA.
Conclusion
Our findings indicate that IL-6 is a strong inducer of vascular calcification and that STAT3 binding and decreased H3K9me3 to Runx2 is associated with IL-6-dependent vascular calcification.

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Association of circulating dipeptidyl-peptidase 4 levels with osteoporotic fracture in postmenopausal women

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Evidence indicates that dipeptidyl-peptidase 4 (DPP4) plays a distinct role in bone metabolism. However, there has been no report on the association, if any, between circulating DPP4 levels and osteoporosis-related phenotypes. This study aimed to determine if DPP4 predicts osteoporotic fracture (OF) risk in postmenopausal women. This case–control study was conducted in multiple centers in Korea. We enrolled 178 cases with OF and 178 age- and body mass index-matched controls. OF was assessed by an interviewer-assisted questionnaire and lateral thoracolumbar radiographs. Bone turnover markers (BTMs), bone mineral density (BMD), and plasma DPP4 levels were obtained in all subjects. After adjustment for potential confounders, subjects with OF had significantly higher plasma DPP4 levels than those without ($P = 0.021$). Higher DPP4 levels were significantly associated with higher levels of all BTMs, but not with BMD at all measured sites. The differences in DPP4 levels according to OF status disappeared after an additional adjustment for each BTM, but not after adjustment for any BMD values. The risk of OF was 3.80-fold (95% CI = 1.53–9.42) higher in subjects in the highest DPP4 quartile than in those in the lowest quartile after adjustment for potential confounders, including

femoral neck BMD. Finally, mediation analyses demonstrated that bone turnover explained about half of the relation between DPP4 and OF. In conclusion, DPP4 may be associated with OF by partially mediating the bone turnover rate. The circulating DPP4 levels may be a potential biomarker that could increase the predictive power of current fracture risk assessment models.

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Insulin resistance independently had the negative association with the bone mineral density in Korean young women

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The relationships between insulin resistance and BMD are not clear. Therefore, we conducted a cross-sectional study to examine the relationship between insulin resistance and BMD among Korean young adult population. This study is based on the Korea National Health and Nutrition Examination Survey (KNHANES) from 2008 to 2010. BMD and body composition were measured by DXA method. Insulin resistances were obtained by HOMA-IR equation. The relationship between BMD and HOMA-IR were analyzed with multiple regression models, which were adjusted with age, body weight, alcohol drink, exercise level and 25(OH) vitamin D level.

We analyzed 3686 persons (men: 1750, women: 1936) who were aged between 20 and 39 years old. HOMA-IR Showed positive correlations with lumbar BMD and femur BMD in men and all BMD in women. However we adjusted with age body weight, alcohol drink, exercise level and 25(OH) vitamin D level, insulin resistance had negative relationship with Lumbar BMD ($B = -0.123$, $P < 0.001$), femur neck BMD ($B = -0.057$, $P = 0.01$).

In this study, BMD were decreased with the increase of insulin resistance in Korean young adult.

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Low bone mass density is associated with tooth loss in postmenopausal women: a nationwide representative study

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Background

Both osteoporosis and tooth loss are major health problems that are frequently observed in postmenopausal women. Authors in this study investigated the relationship between low bone mineral density and edentulism.

Methods

This cross-sectional study used data from the fifth Korea National Health and Nutrition Examination Survey 2011–2012. A total of 2129 postmenopausal women (50–80 years), who had bone mineral density measured and had undergone dental examination. All participants had dental examination by a dentist and were examined for the number of remaining teeth, dental prosthetics, dental implants, and periodontal diseases. In addition, the participants self-reported their oral health behaviours. Multivariate logistic regression via complex sampling was used to estimate odds ratio (OR) for osteopenia and osteoporosis regarding to eight or more tooth loss.

Results

Average number of tooth loss in the participants with normal BMD was significantly lower than those with osteopenia and osteoporosis (4.5 ± 0.4 vs 6.7 ± 0.3 vs 10.4 ± 0.2, P value < 0.001). Women with osteoporosis in femoral neck had a higher risk for eight or more of tooth loss compared to the women with normal BMD in femoral neck (OR, 2.37; 95% CI, 1.88–2.99). Similarly, the subjects with osteoporosis in lumbar spines had a higher risk for eight or more tooth loss (OR, 1.89; 95% CI, 1.52–2.36) in comparison with the group with normal BMD in lumbar spines.

Conclusions

Excessive edentulism was significantly associated to low BMD in postmenopausal women. Persistent dental care is suggested to prevent tooth loss.

Practical implications

Dentists should be aware that postmenopausal women with osteopenia or osteoporosis should have regular dental examinations to prevent tooth loss.

Key Words: bone density, osteoporosis, tooth loss, postmenopausal women

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Impaired quality of life and muscle function in patients with hypoparathyroidism/hypothyroidism compared with only hypothyroidism and controls

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Total thyroidectomy causes postsurgical hypothyroidism (HypoT). Besides HypoT, patients may also develop hypoparathyroidism (HypoPT).

The aim of the study was to assess Quality of Life (QoL), muscle function, and bone indices in patients with postsurgical hypothyroidism and hypoparathyroidism (HypoT + PT) as compared to patients with postsurgical HypoT and healthy controls.

Age and gender matched patients on treatment for HypoT + PT and HypoT were recruited from our out-patient clinic. Matched healthy controls were recruited from the general background population.

Compared with controls, HypoT was associated with a significantly lower mental summary score, whereas patients with HypoT + PT had a significantly lower physical summary score (Short Form 36 Health Survey questionnaire version 2). Moreover, the physical component score was significantly lower in patients with HypoT + PT compared with HypoT. WHO-5 well-being index was significantly lower in both groups of patients compared with controls, but did not differ between groups of patients. Compared with controls, muscle strength and maximal force production was significantly reduced in HypoT + PT, but not in HypoT. In HypoT + PT, the time spend on the *Timed Up and Go test* and the *Repeated Chair Stands test* were significantly longer than in the HypoT- and the control-group. Biochemical markers of bone turnover were decreased in HypoT + PT and bone mineral density was increased at the lumbar spine. However, trabecular bone score (TBS) did not differ between groups.

Postsurgical HypoT + PT is associated with a more severe impairment of QoL than HypoT in particular regarding physical functioning and HypoT + PT patients are hampered by impaired muscle function. Studies on how to improve well-being and muscle function in HypoT + PT patients are warranted.

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Type 2 diabetes mellitus increase the risks of incident osteoporotic fractures in Chinese postmenopausal women: a 5-year prospective study

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Purpose

The aim of this 5-year prospective study was to investigate the association between type 2 diabetes mellitus (T2DM) and incident osteoporotic fracture (IOF) in Chinese postmenopausal women aged 50 years or older.

Methods

We included 826 subjects, participating in both the baseline (2007–2008) and follow-up (2012–2013) studies. Based on the diagnosis of T2DM, subjects were first individualized into Non-T2DM and T2DM groups, which were then categorized, based on the history of previous osteoporotic fractures (POFs) before baseline, into four subgroups: Non-T2DM + Non-POF, Non-T2DM + POF, T2DM + Non-POF and T2DM + POF. Risks of IOFs and baseline values of bone mineral density (BMD) and bone turnover markers (BTMs), were compared between groups. Based on the fracture site, IOFs were further classified into major osteoporotic fracture (MOF) and non-MOF, incidences of which were also compared.

Results
Diabetic subjects, especially those without POF, had significantly lower levels of BTMs but non-decreased values of BMDs than non-diabetic subjects. Comparing to Non-T2DM group, fracture incidence was increased 1.773-fold (95%CI, 0.887–3.544) for IOF and 4.091-fold (95%CI, 1.653–10.120) for Non-MOF in T2DM group. Comparing to Non-T2DM + Non-POF subgroup, fracture incidence was increased 2.561-fold (95%CI, 1.108–5.922) for IOF and 5.595-fold (95%CI, 1.883–16.621) for Non-MOF in T2DM + Non-POF subgroup. Although no statistical significance existed, fracture incidence of T2DM + Non-POF subgroup was comparable to Non-T2DM + POF subgroup, and T2DM + POF subgroup had a higher fracture incidence than Non-T2DM + POF subgroup. Low BTMs were not found significantly associated with high incidence of IOFs in T2DM + Non-POF subgroup.
Conclusions
T2DM was such an important risk factor as previous factures for incident osteoporotic fractures in Chinese postmenopausal women. Except for bone turnover, there might be additional mechanisms contributing to the increased incidence of IOFs in type 2 diabetic patients.

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P431

Bisphosphonate therapy in Langerhans cell histiocytosis: an international retrospective descriptive study

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Introduction
Langerhans cell histiocytosis (LCH) is a monoclonal disorder characterized by proliferation and accumulation of atypical Langerhans cells. Bone involvement is particularly destructive and to date, no standard of care exists. Bisphosphonates are osteoclast inhibitors that could target the multinucleated giant cells within the LCH lesions and might be used to alleviate bone pain and the progression of disease.
Objective
To evaluate the efficacy and safety of bisphosphonates in treating bone LCH and extra-osseous disease.
Methods
An international, multicenter, retrospective study was conducted in children and adults with LCH who received bisphosphonates between 1995 and 2014.
Results
Eighteen patients with single-system or multi-system LCH were identified from 4 centers. All received bisphosphonates therapy either at diagnosis or at $\geq$1st reactivation. Median age at start of bisphosphonates was 23.7 years (range 5.7–38.3), and median follow-up time post bisphosphonate therapy was 2.8 years (range 0.9–5.0). The majority of patients received zoledronate ($n=10$), followed by pamidronate ($n=4$) and alendronate ($n=3$); one patient received both pamidronate and zoledronate. All patients reported significant reduction in pain, to either no or mild pain after administration of bisphosphonates. Thirteen of 18 patients (72%) achieved complete remission (CR) in the bone lesions, including lesions in skin ($n=1$), lung ($n=1$) and pituitary ($n=1$); two had partial response and three had no response. Among the 13 CR patients, 12 had no active disease for a median of 4.1 years (range 2.8–5.1) and one developed radiographic neurodegeneration after 2 years. Bisphosphonates were well tolerated. Progression-free survival (PFS) was $75\pm11\%$ at 3 years, with a trend favoring better PFS ($P=0.24$) in patients with no or first reactivation compared with the rest.
Conclusion
Bisphosphonates are well-tolerated medications that can significantly improve bone pain in patients with osseous LCH lesions, and may be effective in treating extra-osseous disease.

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Differing mechanisms of mineralisation in vascular smooth muscle cells and osteoblasts

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Vascular calcification (VC) involves hydroxyapatite deposition in the arteries and cardiac muscle. VC is thought to share some outward similarities to skeletal mineralisation and has been associated with the transdifferentiation of vascular smooth muscle cells (VSMCs) to an osteoblast-like phenotype. We have previously shown that ATP, UTP and synthetic ATP-analogues (α, β-meATP, β, γ-meATP, Bz-ATP) ($\geq$1 μM) act to potently inhibit both bone mineralisation and VC by $\leq$95%. This study compared the mechanisms by which extracellular nucleotides block these processes. Primary mouse VSMCs and osteoblasts were cultured for up to 21 days in calcifying (2.5 mM phosphate) and osteogenic (2 mM β-glycerophosphate, 50 μg/ml ascorbate) medium, respectively. Cells were treated with 1–100 μM ATP, UTP α, β-meATP, β, γ-meATP or Bz-ATP for the duration of the culture. Basal alkaline phosphatase (TNAP) activity was 12-fold higher in mineralising osteoblasts compared to calcifying VSMCs ($P<0.001$). The activity of NPPs, which generate pyrophosphate from ATP, was tenfold higher in calcifying VSMCs ($P<0.001$). In differentiating and mature osteoblasts, extracellular nucleotides ($\geq$10 μM) inhibited TNAP activity by $\leq$50% ($P<0.001$). In contrast, ATP, UTP and ATP-analogues stimulated TNAP activity in calcifying VSMCs by $\leq$20-fold ($P<0.001$); at these concentrations VC was typically reduced by $\leq$90%. Prolonged treatment with ATP and related compounds ($\leq$100 μM) had no effect on osteoblast number and viability. However, in calcifying VSMCs treatment with extracellular nucleotides increased cell number by $\leq$2.5-fold and reduced the percentage of dead cells by $\leq$70% ($P<0.001$). Since VC is also associated with increased apoptosis, extracellular nucleotides may prevent VC by promoting VSMC survival. Our results indicate that although the functional effects of extracellular nucleotides on bone mineralisation and VC are similar, the underlying cellular mechanisms are distinct. Notably it suggests that TNAP may not exert significant effects on VC. Our findings indicate that, despite outward similarities, there are key differences between the processes of bone mineralisation and vascular calcification.

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P433

Genetic and clinical characteristics of Chinese pseudohypoparathyroidism patients

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Objects
Seventy seven clinically diagnosed pseudohypoparathyroidism (PHP) patients from our hospital during 2000–2010 were recruited to analyze the clinical features and molecular genetics of Chinese PHP patients.
Methods
The clinical data of the 77 PHP patients were retrospectively analyzed. Methylation status of *GNAS* was detected by combined bisulfiterestriction analysis. Genome DNA was extracted from peripheral blood lymphocytes. *GNAS* gene mutation was screened by PCR and Sanger sequencing. The study was approved by the local Ethics Committee of our center.
Results
There were 17 PHP-Ia patients with normal methylation in all four Differentially Methylated Region (DMRs), 13 familial PHP-Ib patients with loss of methylation in *A/B*, and 47 sporadic PHP-Ib patients with broad *GNAS* methylation changes according to combined bisulfiterestriction analysis. Furthermore, 3.0 kb deletion in *STX16* were identified in nine familial PHP-1b patients with no 4.4 kb deletion in the same locus found. There were three new *GNAS* mutations (c. 314-316 del AAG, ins T c.352 insC, VS12 + 1G > T) identified in PHP-1a individuals. Among all the subjects, the average serum calcium was 1.74 ± 0.29 mM and the mean PTH level was 371.8 ± 223.8 pg/ml at the first visit to our center. AHO phenotype existed in all PHP-1a patients but only 57.4% of sporadic PHP-1b and 44.4% of familial PHP-1b patients respectively. Among the 41 subjects with results of thyroid function, TSH resistance was detected in 6/6 (100%) of PHP-1a, 10/27 (37.0%) of sporadic PHP1b, and 1/6 (16.7%) of familial PHP1b patients.
Conclusion
Clinical presentation may overlap among the three kinds of PHP individuals despite earlier onset and higher incidence of AHO and TSH resistance in PHP 1a,

while the underlying molecular genetics are totally different. Thus careful molecular and epigenetic analysis are necessary to make the right diagnosis.

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P434

Cyclophilin B deficiency is associated with defective differentiation of bone cell populations and bone hypermineralization

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Deficiency of Cyclophilin B (CyPB) causes recessively inherited Type IX osteogenesis imperfecta, a moderately severe to lethal bone dysplasia. CyPB, encoded by PPIB, is an ER-resident peptidyl-prolyl *cis-trans* isomerase (PPIase) that catalyzes the rate-limiting step in collagen folding, and also functions as a component of the collagen prolyl 3-hydroxylation complex. We previously demonstrated in a $Ppib^{-/-}$ mouse model that CyPB PPIase activity regulates collagen lysyl hydroxylation, crosslinking and fibrillogenesis, which contribute to maintaining bone mechanical properties. The roles of CyPB in the differentiation and function of specific bone cell populations are unknown. Our screening of whole femora revealed significantly decreased expression of genes associated with osteogenesis in CyPB-deficient mice versus wild-type, including *Runx2* (−30%), *Rankl/Opg* (−55%) and Ibsp (−23%), respectively. In differentiated $Ppib^{-/-}$ calvarial osteoblasts in culture, expression of osteocyte markers was increased, including *Mepe* (+125%), *Dmp1* (+150%) and *Sost* (+250%). Osteocyte lacunae from 2 month $Ppib^{-/-}$ femora were also abnormal in histologic and 2D analyses of serial qBEI cross-sections, revealing significantly increased density of lacunae with a higher proportion of lacunae having smaller areas, versus wild-type. Most interestingly, $Ppib^{-/-}$ osteoclasts revealed an intrinsic maturation defect. In $Ppib^{-/-}$ femora, both *Acp5* (Tracp) and *Ctsk* (Cathepsin K) transcripts associated with osteoclast numbers and activity were reduced 38–39%, consistent with reduced *Rankl/Opg* versus wild-type. RANKL-induced maturation of osteoclasts from $Ppib^{-/-}$ femoral marrow was severely reduced, with a nearly complete absence of multinucleated cell formation, compared to wild-type. Moreover, qBEI revealed a shift of the bone mineralization density distribution towards higher mineral content, with a concominant decrease in the heterogeneity of mineralization. These data suggest that absence of CyPB alters maturation and function of both osteoblasts and osteoclasts, resulting in a decreased formation and turnover state contributing to bone hypermineralization. The mechanisms by which CyPB regulates bone cell populations are currently under investigation.

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P435

Association between serum levels of PPARγ and vertebral fractures in type 2 diabetes mellitus patients

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Introduction
Type 2 diabetes mellitus (T2DM) is a risk factor for the development of fractures. Several studies have shown an inverse relationship between osteoblastogenesis and adipogenesis through a competition model between these processes.
PPARγ acts as regulator of adipogenesis and its increased expression is associated to decreased osteoblastogenesis. The treatment of insulin resistance with glitazones, one of the ligands of PPARγ, reduces bone mineral density increasing risk of fractures. Thus, T2DM patients may have higher levels of PPARγ inhibiting bone mineralization and increasing adipogenesis and bone fragility.
Objectives
To examine serum levels of PPARγ in T2DM patients without treatment with glitazones, checking endogenous serum PPARγ expression in patients with osteoporosis and vertebral fractures (VF).
Methods
Cross-sectional study including 75 patients with T2DM with presence/absence of osteoporosis and morphometric VF. Anthropometric and biochemical parameters, calciotropic hormones, bone turnover markers (MRO) and bone mineral density (BMD) were evaluated. PPARγ levels were determined by ELISA techniques (Cusabio).
Results
No significant differences of serum PPARγ concentrations were observed according to the presence/absence of osteoporosis ($P=0.208$). Neither, correlation between PPARγ levels, MRO nor BMD values was observed ($P>0.05$). However, circulating levels of PPARγ were significantly higher in the T2DM group with VF compared to patients without VF ($P=0.001$). In a logistic regression model including age, sex, sedentary lifestyle, family history of fracture, vitamin D levels and HbA1c as independent variables, only PPARγ levels were independently associated with the presence of morphometric VF (OR 1.002 (95% CI 1000–1004); $P=0.018$) indicating an increase of 2% in fracture risk per pg/ml of increased PPARγ.
Conclusions
Serum levels of PPARγ are increased in T2DM patients with fractures suggesting that this receptor might be involved in the regulation of mineral metabolism and bone fragility in T2DM.

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P436

Determinants of health related quality of life in adults with osteogenesis imperfecta

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Osteogenesis imperfecta (OI) is a systemic connective tissue caused by mutations in collagen type 1 related genes. Patients with OI suffer from multiple fractures and various degrees of growth deficiency and bone deformity. Other symptoms are early hearing loss, abnormal dental tissue and hypermobility. It is not known whether the systemic effect of a defect collagen type 1 influences the quality of life in people with OI. We aimed to investigate health related quality of life (HRQoL) in a well characterized cohort of adults with OI, and hypothesized that there was no difference in HRQoL in adults with mild, moderate and severe OI compared with an adult population without OI.
We examined 85 adult patients with mild to severe OI and obtained information physical and mental HRQoL by the SF-36 questionnaire, and compared the data to investigations of HRQoL in the general population.
All patients with OI, regardless of severity, had significantly lower mean scores in domains describing physical HRQoL and lower mean physical component scores compared to the general population (Table 1). In addition, patients with severe OI had the lowest mean score on physical HRQoL. On the contrary mental health score did generally not differ between OI groups or compared to the general population.
OI has an impact on physical HRQoL. The scores on physical health were correlated to severity of the OI disease. Surprisingly, the mental scores in the OI patients were generally comparable to a normal reference population.

Table 1.

OI severity	Mild	Moderate	Severe	Without OI
PCS (±S.D.)	40.5±11.5*	39.2±11.5*	30.5±9.4*,†	51.2±8.8*
MH (±S.D.)	79.9±15.9	78.9±21.5	82.2±10.3	81.8±15.5
MCS (±S.D.)	52.7±10.3	53.0±12.5	60.6±8.7*	54.0±8.4

PCS, physical component score; MH, mental health; MCS, mental component score; *$P<0.05$ (OI vs non-OI). †$P<0.05$ (severe OI vs mild/moderate OI).

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P437

Evaluation of targeted next-generation sequencing in diagnosis of Chinese adult-onset idiopathic hypoparathyroidism

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Objectives
Several genes have been recognized to be associated with nonsurgical hypoparathyroidism. Most previous studies focused on gene mutation among paediatric hypoparathyroidism patients. Data about gene mutations in adult-onset hypoparathyroidism patients is still lacking. This study was designed to evaluate the role of gene defects in the pathogenesis of adult-onset hypoparathyroidism in China through the targeted next-generation sequencing (NGS).
Subjects and methods
We recruited 17 patients with adult-onset nonsurgical hypoparathyroidism who were regularly followed or newly diagnosed at our centre during the past one year. Nine of them developed hypercalciuria during the treatment with calcium and vitamin D agents. None of them had physical deformity or family history of hypoparathyroidism. Targeted NGS was performed to screen 10 related genes, including *AIRE, AP2S1, CASR, CLDN16, FAM111A, GATA3, GCM2, PTH, TBCE* and *TRPM6*.
Results
A novel homozygosis mutation of *GCMB* gene [c.130G > A (p.G44S)] was identified which was predicted to be deleterious by PolyPhen2. The patient was a 36-year-old woman who suffered from paroxysmal carpopedal spasms in the menstrual cycle for ten years. Before treatment, the serum calcium and phosphorus was 1.48 mmol/l and 2.29 mmol/l, respectively. And the PTH concentration was lower than 3.0 pg/ml. intracranial calcification and cataract were also identified. During treatment with calcium and vitamin D, she developed hypercalciuria when her serum calcium reached 2.04 mmol/l. Hydrochlorothiazide was administered. No evidence of urolithiasis was found.
Conclusions
In this study, we identified the genetic defect only in 1 patient (5.9%). In adult-onset nonsurgical hypoparathyroidism without other diagnostic clues, the gene mutation screening as the first choice to clarify the aetiology was not recommended.

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P438

DXA in clinical practice: invest in quality to improve accuracy and clinical relevance

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Introduction
Osteopetrosis is a disorder with diminished bone resorption due to osteoclastic abnormality resulting in hard and brittle bones. Subtrochanteric area is quite susceptible to fractures because this is an area of high stress. Operative intervention has a high rate of perioperative complications.
Methods and results
We present a series of four Subtrochanteric fractures treated surgically in three patients of Osteopetrosis in a tertiary level Orthopaedic centre over the last 3 years. The patients had a mean Harris Hip Score of 85, at a mean follow up of 21.5 months; the fractures united and the patients successfully returned to their normal activities of daily living.
Conclusions
We concluded that operative fracture treatment in patients with osteopetrosis is difficult; but a good preoperative planning minimises the duration of the operation and the risk of intra-operative complications. Our series provides evidence that with operative treatment these patients can return to to normal function successfully.

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P439

Quality of life and psysical activicy in patients with non-surgical hypoparathyroidism and pseudohypoparathyroidism

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Background
Non-surgical hypoparathyroidism (NS-HypoPT) and pseudohypoparathyroidism (Ps-HypoPT) are both rare diseases, with a prevalence of 2/100.000 and 1/100.000, respectively.
Studies on Quality of Life, QoL, are sparse, but studies have shown an increased risk of fatigue and depression.
Methods
Using the Danish version of SF36v2 and WHO-5 Well Being Index, we investigated the physical and mental QoL among patients with NS-HypoPT ($n=10$) and Ps-HypoPT ($n=9$). Furthermore we investigated the physical activity using the validated questionnaire, Physical Activity Scale, PAS, measuring time spend sleeping, working and leisure during 24 h.
Results
The SF36v2 shows a significantly reduced score among patients with NS-HypoPT compared to a norm-based population at the subdomains: physical functioning, PF (45.6 ± 10.4), role-physical, RP (43.7 ± 11.6), general health, GH (43.0 ± 11.9), vitality, VT (45.1 ± 13.7) and role-emotional, RE (44.6 ± 12.9, all $P<0.05$). In contrast, patients with Ps-HypoPT did not differ from norm-based controls at any of the domains, and the physical and mental component score did not differ between any of the patient groups and controls. However, NS-HypoPT have a significantly reduced physical component score compared with Ps-HypoPT ($P<0.05$).
The overall WHO-5 Well Being Index score was 52 (IQR 36–72) in the NS-HypoPT group (min 16; max 84), whereas it was 64 (IQR 50–77) in the Ps-HypoPT group (min 44; max 80). A score below 28 indicates depression ($N_{NS\text{-}HypoPT}=3$; $N_{Ps\text{-}HypoPT}=0$), whereas a score between 28–50 suggesting poor emotional well-being ($N_{NS\text{-}HypoPT}=7$; $N_{Ps\text{-}HypoPT}=2$). The rest have a score above 50 suggesting a state of well-being.
The median 24 h metabolic equivalent, MET-value, was 40.6 (IQR 35.4–57.3) in the NS-HypoPT group and 37.8 (IQR 33.9–44.5) in the Ps-HypoPT group ($P=0.31$).
Conclusion
Patients with NS-HypoPT have a significantly reduced QoL compared with both a norm-based population and patients with Ps-HypoPT. On the other hand, patients with Ps-hypoPT do not seem to suffer from impaired QoL.

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P440

Mice lacking periostin are resistant to bone microstructural alterations during lactation

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Periostin is a matricellular protein expressed in late osteoblasts/osteocytes, which levels increase in response to PTH and mechanical loading. In turn periostin contributes to modeling based bone formation while restraining bone remodeling. Periostin is also a substrate of cathepsin K and inhibition of periostin blunts the effects of cathepsin K inhibition on bone. Considering the important role of osteocytes and their cathepsin K expression on osteolysis during lactation, we investigated the contribution of periostin to lactation-induced changes in bone microstructure.
12-week-old $Postn^{-/-}$ and $Postn^{+/+}$ female mice were mated. After birth, lactating females were studied at day 14 of lactation (L), female mice of the same age were used as control ($n=5$–6 per group). Trabecular and cortical microarchitecture of the distal and midshaft femur was evaluated by a standard microCT (ScancoCT40) and porosity by a nanoCT (ScancoCT1172).
In $Postn^{+/+}$ mice, L significantly decreased periostin gene expression (-32% vs non-L, $P<0.05$) and increased cathepsin K, MMP13 and RANKL gene ($+164$, $+138$ and 213% vs non-L, all $P<0.05$). As expected, trabecular bone volume fraction (Tb. BV/TV), Tb. number (Tb.N), cortical bone volume (CtBV), Ct thickness (CtTh) decreased while Ct porosity increased in these conditions (-54, -15, -21, -33 and $+14\%$ respectively, vs non-L, $P<0.05$). In contrast, L had no effect on Tb. BV/TV, Tb.N, Ct.BV and porosity in $Postn^{-/-}$ mice. CtTh

decreased in response to L (−20% vs non-L, $P<0.05$) however significantly less in *Postn*$^{-/-}$ compared to WT mice. Hence a significant interaction between periostin and lactation was found on these parameters ($P<0.05$).
These results indicate that inhibition of periostin expression during lactation, and potentially its degradation by cathepsin K from osteocytes, is an important mechanism for bone loss in these conditions. Whether periostin affects more remodeling and/ or osteocytic osteolysis is currently under investigation.

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Abstract withdrawn.

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Investigation of the Paget's disease susceptibility locus on chromosome 15q24 using targeted next generation DNA sequencing approach

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Paget's disease of bone (PDB) is a common disease characterised by focal abnormalities in bone turnover. Previous GWAS identified a susceptibility locus for PDB on chromosome 15q24 tagged by a coding SNP rs5742915 (p.Phe645Leu) located in *PML*. The aim of this study was to fine map this locus to identify functional genetic variants that predispose to PDB using targeted DNA sequencing and functional analysis in bone cells.
A 200 kb region surrounding the rs5742915 was captured and sequenced on illumina HiSeq2000 platform in 138 PDB cases and 50 controls. Potential regulatory variants were assessed using the ENCODE database. Expression analysis was performed using western blot in cultured bone cells.
DNA sequencing results identified 22 missense variants (five in *LOXL1,* 13 in *PML*, three in *ISLR* and one in *ISLR2*). However, only three missense variants (all located within *PML*) showed significant association with PDB ($P<0.05$). Additionally, 33 potentially regulatory variants were identified in this region, of which two variants located in *PML* promoter showed significant association with PDB ($P<0.003$).
We next investigated PML gene expression in bone cells and found that PML was expressed in RAW 264.7 cells as well as bone marrow derived macrophages and at all stages during their differentiation into osteoclasts. PML was also expressed at all stages of osteoblast differentiation in cultured cells derived from mouse calvaria. PML is a tumour suppressor gene that is involved in multiple cellular functions including cell growth, apoptosis, and antiviral responses but has never been implicated in bone metabolism. It may be involved by controlling maturation of myeloid cells, proliferation and osteogenic differentiation of human mesenchymal stem cells. Our results suggest that *PML* is the susceptibility gene for PDB in this region. However, further work is in progress to confirm our findings in larger cohorts and to investigate the role of PML in bone metabolism using knockdown and over-expression in bone cells.

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Deep characterization of a zebrafish model for dominant osteogenesis imperfecta

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Dominant osteogenesis imperfecta (OI) is a bone disease mainly caused by collagen type I mutations and characterized by bone fragility and growth delay. Nowadays no definitive cure is available. A zebrafish OI model (Chihuahua) carrying an heterozygous G574D substitution in the α1 chain of collagen type I was generated by ENU mutagenesis and is available in our laboratory. Control (WT) and mutant (Chi+/−) fish growth was followed up from day 1 post fertilization to 5 month old. Skeleton was analyzed by X-ray, μCT and specific bone staining. Collagen I was extracted from skin, scales and bone and analyzed by SDS-PAGE. Collagen melting stability was evaluated by Differential Scanning Calorimetry (DSC). Electron microscopy of osteoblasts and confocal microscopy of fibroblasts were also performed. At morphological level, Chi+/− embryos show a typical fin fold bending, whereas at adult age a smaller size compared to WT was significant starting from 3 months. X-ray and μCT revealed reduced mineralization in adult Chi+/− vs WT. Skeletal staining highlighted multiple bone fractures and deformities, such as alterations in neural spine inclination. A delayed mineralization was also observed in mutant larvae. Collagen type I presents a delay in migration and a broadening of the α bands in SDS-PAGE, revealing the presence of overmodification, while DSC showed ∼2 °C reduction in the Tm of mutant collagen compared to WT. The presence of an endoplasmic reticulum (ER) enlargement in osteoblasts and fibroblasts of mutant fish was evident by microscopy analysis. The collagen overmodification and ER retention and the skeletal phenotype found in Chi+/− fish reproduce the main features reported in OI patients, validating this model for the study of the disease and for the evaluation of novel possible therapy.
All animal experiments were in agreement with EU Directive 2010/63/EU.

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Clinical and genetic analysis of multiple endocrine neoplasia type 1-related primary hyperparathyroidism in Chinese: a single-center experience over ten years

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Objective
Multiple endocrine neoplasia type 1-related PHPT (MHPT) differs in many aspects from sporadic PHPT (SHPT). The aims of the study were to summarize

the clinical features of Chinese MHPT and compare the severity of the disease with SHPT.
Design and methods
A total of 40 MHPT cases (27 sporadic, seven families) and 169 SHPT patients of Chinese descent were retrospectively analyzed. X-rays and ultrasound were used to assess the bone and urinary system. Dual energy X-ray absorptiometry (DXA) were performed to measure bone mineral density (BMD). The present study was approved by the local Ethics Committee.
Results
The mean age at diagnosis of PHPT in MHPT group was younger than SHPT group (45.0 vs 50.7 years, $P=0.025$). Compared with SHPT patients, MHPT patients showed lower prevalence of skeletal involvement (87.1% vs 57.5%, $P<0.001$) but higher prevalence of urolithiasis/renal calcification (40.2% vs 60.0%, $P=0.024$). MHPT patients showed higher phosphate level (0.84 vs 0.73 mmol/l, $P<0.05$) but lower ALP (103.0 vs 174.0 U/L, $P<0.001$) and PTH (4.0 vs 9.8×upper limit, $P<0.001$) levels than SHPT patients. There were no significant differences in BMD *Z*-scores at the lumbar spine and femoral neck between the two groups even after adjustment of age, sex, course of PHPT ($P=0.829$, 0.510 respectively). Compared with SHPT, the incidences of parathyroid hyperplasia and multi-glandular involvement were both higher in MHPT patients (both $P<0.001$). *MEN1* gene mutations were detected in 27 MHPT cases. Nine mutations were novel, and one of which was deletion of exon 5 and 6.
Conclusions
MHPT patients showed more common kidney complications but less common skeletal involvement despite a milder biochemical presentation compared with SHPT patients. *MEN1* mutation detection rate was 79.4% and nine mutations were novel.

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P446

Impacting factors of serum 25-hydroxyvitamin D levels of elderly in community in Shanghai

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Objective
To analyse the influential factors of lifestyle on vitamin D status among aged people in Shanghai community.
Method
Lifestyles of 3846 elderly men and women aged over 65 years were investigated using a self-designed questionnaire from ten communities of urban and suburb in Shanghai China, which contained sociodemographic factors and lifestyle factors and so on. The levels of serum 25(OH)D were measured by Cobas Diagnosis System of Roche. Univariate analysis was used to select the impactor factors on 25(OH)D levels at first, and then these factors were tested by logistic multiply regression to eliminate the interaction, and finally found the significantly important factors.
Results
The prevalence of vitamin D deficiency was 44.02% and insufficiency was 38.35% in our study cohort. Serum 25(OH)D levels showed a relatively skewed distribution (median 22.73 ng/ml and mean 24.1 ng/ml for men; and median 19.99 ng/ml and mean 21.0 ng/ml for women). Logistic multiple regression showed that education levels, outdoor physical activities, calcium and vitamin D supplements and residence were statistically significant ($P<0.05$) on 25(OH)D level of elderly men, while drinking milk or not, calcium and vitamin D supplement, BMI and residence were statistically significant on 25(OH)D level of elderly women. To old men,physical activity ($\geq$30 min/day) was protective factor while uneducated (less than primary education) was risk factor, and to old women,milk consumption ($\geq$250 ml/day) was protective factor while BMI$>$ 26.06 kg/m^2 was risk factor, however, calcium supplements and suburban residence were protective factors for both men and women.
Conclusions
Serum 25(OH)D concentration was closely related to lifestyle of aged people in community. Calcium and vitamin D supplements, suburban residence were protective factors, and drinking milk and outdoor physical activities improved serum 25(OH)D levels. Scientific lifestyle is essential for aged people to improve the health-related quality of life.

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P447

The characteristics of bone mineral density in men of different ages with type 2 diabetes

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Objective
To investigate the characteristics and related factors of bone mineral density (BMD) in men of different ages with type 2 diabetes.
Methods
Four hundred and forty four male patients diagnosed with type 2 diabetes and 208 healthy men were involved from 2013 to 2014. The BMD of all men including lumbar spine (L1–L4), femoral neck, trochanter, and total hip were detected using dual-energy X-ray absorptiometry produced by the US company. We grouped the patient with T2DM by every 10 years old and compare their BMD. And we also grouped the healthy men and patients by every 5 years old. Then we compared their differences. The correlation analysis of BMI, duration of diabetes, creatinine, serum calcium, phosphorus, the level of lipid and BMD was performed.
Results
i) The BMD of neck and Ward's triangle are different among different age with T2DM ($P<0.05$). ii) The BMD of Ward's triangle between the T2DM one and the healthy one are different in 40–44 (0.737 ± 0.255 vs 0.582 ± 0.162) ($P<0.05$), while there is no difference in 45–59. iii) The BMD of L1 with T2DM was positively correlated with BMI, Cr and Ca ($r=0.258$, 0.132, 0.108). The BMD of L2, L3 was positively correlated with BMI, Cr (L2 $r=0.189$, 0.094 vs L3 $r=0.123$,0.105). The BMD of L4 was positively correlated with BMI ($r=0.115$). The BMD of torch, neck, Ward's triangle, Htot were positively correlated with BMI, TC, Ca and P, while they were negatively correlated with age and duration ($P<0.05$).
Conclusion
It shows that the BMD between T2DM and healthy people is different when matched with age. There are many factors for men with T2DM to have influence on BMD in different parts., especially BMI, age and duration. We should pay more attention to the men with type 2 diabetes who are more than 50 years old in case of osteoporosis.

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P448

The characteristics and related factors of vascular calcification(VC) in lower limbs of patients with type 2 diabetes

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Objective
To investigate the characteristics and related factors of vascular calcification (VC) in lower limbs of patients with type 2 diabetes.
Methods
204 patients diagnosed with type 2 diabetes had suffered from the symptoms on lower limb and took ABI and CTA test. We collected their basic information and also study their biochemical index. VC was defined when the ABI >1.3 of posterior tibial artery and dorsalis pedis artery, while we scored VC on CT scanning as follows: score 0, no calcification; score 1, the grey value is >130 HU and the area is >1 mm^2. Each vessel was divided into eight parts on CTA imaging. And we analysis the data.
Results
1) Both sides of lower limb have VC with different degrees. The percentage in left and right side of the lower limbs were 92.7, 89.3, 73.6, 75.3, 66.9, 66.3, 46.1, 49.4, 61.2, 62.4, 60.7, 59, 43.3, 44.4, 39.9, 39.9% respectively ($P>0.05$).
ii) Calcification score has positive correlation with age, diabetes duration, creatinine, urine trace albumin, ACR and GFR ($\rho=0.558$, 0.301, 0.242, 0.282, 0.246, 0.233) ($P<0.01$). And it has negative correlated with triglycerides and Ca × P product ($\rho=-0.186$, -0.155) ($P<0.05$).
iii) With hypertension, people would got a higher VC score (HBP 10(5, 14) vs NHBP 6 (1, 11)) ($P<0.01$).
iv) VC cases of left and right posterior tibial artery and dorsalis pedis artery were 19, 13, 6, 7 respectively by Doppler, while the cases were 108, 105, 71 and 105 through CT. It is different for VC by ABI and CT ($\chi^2=67.71$, 76.95, 56.33, 53.89) ($P<0.01$).

Conclusion
i) It is easier to get VC for people with diabetes in lower limb symptoms. It is mainly distributed in the artery above knee. People with hypertension will increase the risk of calcification and get a poor prognosis.
ii) Age, duration and ACR are independent risk factors for VC.
iii) CT angiography has higher accuracy for the diagnosis of VC than Doppler.
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P449

MSC injection modulates osteocyte number after high dose single limb irradiation in a murine model

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Introduction
In accidental irradiations, skin, muscle and bone are altered. Mesenchymal Stem Cell (MSC) treatment has already been used for skin healing in accidental irradiations, but its effects on irradiated bone are still unknown.
In this study, we characterised a mouse model of high dose local irradiation of bone and the effects of MSCs injections on osteoradionecrosis.
Methods
Nude mice were locally irradiated in the left tibia, at a high dose (120 Gy). One week after irradiation, the mice were injected with 1.10^6 MSCs from bone marrow, systemically, or inside the medullary of the irradiated tibia. PBS was injected in control mice.
Bone microarchitecture was analysed by μCT scanning and bone strength by nanoindentation. The vascular network was observed using barium sulfate perfusion. Bone fibrosis and osteocyte number were quantified on sirius red and toluidine blue stained histological images.
Results
We observed an increase of BV/TV, Tb.Th and Ct.Th in the irradiated leg compared to control leg as early as 15 days after irradiation. Collagen fibrotic content was decreased in the bone matrix, and increased in the bone marrow. The percentage of full lacunae in bone matrix was also decreased. Bone hardness was reduced in the irradiated bones after 1 month. Finally, we observed a reduction in blood vessel perfusion in the irradiated bone.
Interestingly, in the MSC treated mice, the percentage of full lacunae was higher than in PBS controls but remained lower in the irradiated leg than in contralateral one.
Conclusion
We demonstrate here that a high dose local irradiation strongly alters bone: we observed an early increase of matrix thickness associated with a decrease in collagen density and osteocyte number, leading to a weakening of bone. MSC injection has positive effects on the number of full lacunae demonstrating a promising potential of this treatment.
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P450

Cortical density is lower in patients with Graves' disease compared to healthy controls

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Objective
Hyperthyroidism leads to reduced mineral bone density and is associated with increased risk of fracture. Whether the effect of increased bone turnover is identical in cortical and trabecular bone in unknown. Using a cross sectional design we aimed to investigate which bone compartment is affected.
Methods
Nineteen newly diagnosed patients with Graves' disease (GD) and nineteen euthyroid controls matched on age, gender and menopausal status were scanned using a high-resolution pQCT scanner (XtremeCT, Scanco Medical AG, Bruüttisellen, Switzerland). Volumetric bone mineral density, cortical and trabecular architecture, and strength as estimated by finite element analysis were measured in the distal radius and tibia.
Result summary

		Cortical bone density [2] (mg HA/cm^3)	P^1	Cortical thickness[2] (mm)	P^1
Radius	Controls	840 (804–875)	0.037	0.60 (0.55–0.66)	0.085
	Patients	883 (851–929)		0.77 (0.60–0.93)	
Tibia	Controls	871 (833–895)	0.002	1.05 (0.91–1.29)	0.065
	Patients	903 (881–927)		1.22 (1.11–1.35)	

[1]Mann Whitney *U*-test for difference between groups.
[2]Median with interquartile range.
Age was similar between groups with a mean age of 39 in patients and 38 in controls. No significant difference in trabecula architecture or strength was found in patients compared to controls.
Conclusion
Loss of bone density at distal sites in patients with GD seems caused by reduced cortical density.
Whether this is the major cause for the increased risk of fractures in GD needs further explorations.
Ethical approval was obtained from all required authorities.
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P451

Rankl$^{-/-}$ mesenchymal stromal cells have an unexpected osteogenic differentiation defect which is improved by a RANKL-expressing lentiviral vector

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Osteoclast-poor RANKL-dependent autosomal recessive osteopetrosis (ARO) is a rare bone disease characterized by an increase in bone density due to the failure of bone resorption by impaired osteoclast formation. Haematopoietic stem cell transplantation is not an effective therapy for this ARO form, since in bone RANKL is produced mainly by cells of mesenchymal origin. Whether also these cells, besides the osteoclast, are in some way affected by RANKL deficiency is not known. To verify this, we established and characterized bone marrow derived Mesenchymal Stromal Cells (BM-MSC) from the Rankl$^{-/-}$ (KO) mouse model, which recapitulates the human disease, and from WT mice. No differences in morphology, immunophenotype and proliferation capacity were found between KO and WT MSC. However, KO MSC displayed a reduced clonogenic potential with a decrease of stemness genes expression. KO MSC were able to normally differentiate towards the adipogenic and chondrogenic lineages, while displayed a significantly impaired osteogenic differentiation capacity compared to the WT MSC, as demonstrated by reduced Alizarin Red staining (ARS) and expression of osteogenic genes. To confirm that this alteration was due to the lack of functional RANKL, we corrected the genetic defect by transducing KO MSC with a third generation lentiviral vector expressing human soluble RANKL (hsRL). In corrected MSC hsRL production and secretion was measurable, stable over time and comparable to sRL levels in WT MSC. KO MSC stably expressing hsRL showed an improved osteogenic differentiation capacity compared to the KO MSC, as demonstrated by increased ARS and expression of osteogenic genes. The expression of RANK receptor in both MSC suggested an autocrine role of sRL as

possible mechanism. Overall, the gene therapy approach to restore hsRL expression ameliorated KO MSC functionality and could be considered for an MSC-based therapy to treat RANKL-dependent ARO.

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P452

Micro-RNA expression profiling in Paget's disease of bone

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Since their initial discovery, microRNAs (miRNAs) have emerged as critical post-transcriptional regulators of gene expression that are able to modulate bone remodeling. Nonetheless, despite the peculiar and aggressive phenotype of pagetic osteoclasts and the associated increase in osteoblast activity, whether deregulation of miRNAs is involved in Paget's disease of bone (PDB) remains unknown. Here, we performed a serum miRNA expression profile (Taqman Human MicroRNA Array Card Set v3.0) in peripheral blood mononuclear cells (PBMCs) from 20 PDB patients (ten with and ten without Q16STM1 mutation) and 20 age-matched subjects with or without osteoporosis. Data from Array Cards were exported using ViiA7 RUO software and then analyzed using Expression Suite Software v1.0.1 (Applied Biosystem). All values were normalized using different housekeeping miRNAs. Overall, 22 miRNAs were significantly upregulated in PBMCs from PDB cases, with a fold change above three (miRNAs-31, -32, -124a, -132, -182, -221, -339, -345, -410, -451, -485.3p) or between 2 and 3 (miRNAs-19a, -30b, -30c, -27a, -125a, -146a, -148a, -200c, -223, -301, -365) than in non-pagetic controls. For most of these miRNAs (miRNAs-19a, -27a, -30c, -32, -125a, -132, -200c, -221, -223, -301, -345, -365, -410, -485.3p) the association was significantly higher in carriers of Q16STM1 mutation. Moreover, seven miRNAs (miRNAs-124a, -132, -148a, -182, -200c, -221, -451) were also significantly upregulated in osteoporotic than non-osteoporotic subjects. Taken together, eight of these miRNAs (miRNAs-26a, -30c, -31, -124a, -146a, -148a, -182, -223) were previously related with bone homeostasis. In particular, while the increase in miRNAs146a, -148a and -223 is consistent with their pro-osteoclastogenic effects observed *in vitro* by previous studies, and might account for the increase in osteoclast activity of PDB, the upregulation of miR-26a and -124a was unexpected, given their role as suppressors of osteoclastogenesis, possibly reflecting counteracting mechanisms on osteoclasts precursors. Conversely, miRNAs-30c and -182 have been mainly related to osteoblastogenesis and the regulation of bone formation. Further studies are required to better address the mechanisms leading to miRNAs deregulation and assess the role of the identified miRNAs as potential biomarkers or therapeutic targets in PDB as well as in other disorders of bone metabolism.

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P453

Two novel mutations of RUNX2 gene in two sporadic cleidocranial dysplasia patients

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Background
Cleidocranial dysplasia (CCD) is an autosomal-dominant skeletal dysplasia syndrome characterized by delayed closure of cranial sutures, remained-open fontanels, hypoplastic clavicles, abnormal dentition including retention of the primary teeth and delayed eruption of secondary dentition, and short stature. The responsible gene for CCD is *RUNX2*, which encodes an important transcription factor for osteoblast development and bone formation. CCD is caused by the haploinsufficiency of RUNX2.
Objective
To analyze the clinical manifestations and the mutation of *RUNX2* gene in two CCD patients.
Methods
Clinical data and peripheral venous blood samples were collected from two patients. Genomic DNA was extracted from peripheral blood samples, Polymerase Chain Reaction(PCR) and Sanger sequencing were performed to analyze the possible mutation of *RUNX2* gene.
Results
The two patients appear with typical CCD phenotypes involving clavicular hypoplasia, patent fontanels, dental abnormalities and short stature. Sanger sequencing revealed two novel heterozygous mutations of *RUNX2* gene in two patients. One nonsense mutation c.1123C>T (Q375X) in patient 1, another is a frameshift mutation 1126delT (F376S) in patient 2, both cause a premature termination of translation and an truncated protein of RUNX2.
Conclusion
RUNX2 is the pathogenic gene for CCD. Here we identified two unreported mutations of *RUNX2* gene in two CCD patients, which elucidated the molecular mechanism for their CCD phenotypes and further enrich the mutation spectrum of *RUNX2* gene.

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P454

Bone mineral density and vascular calcification in obesity

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Background
The presence of vascular calcification (VC) is a predictive factor for the development of cardiovascular diseases, especially in the obese population. VC has also been inversely associated with bone mineral density (BMD) but the results have been inconsistent. The main aim of this study was to evaluate the associations between VC, obesity and volumetric BMD (vBMD).
Methods
We studied 148 healthy men and women, aged 55–75 years, divided into three groups based on their BMI: normal weight (BMI 18.5–25 kg/m^2 $n=58$), overweight (25–30 kg/m^2 $n=30$) and obese (>30 kg/m^2 $n=60$). vBMD of the distal tibia and radius was measured by high-resolution peripheral quantitative computed tomography (HR-pQCT). Quantitative computed tomography (QCT) was used to determine vBMD of the lumbar spine (L1–L3) and proximal femur. Radius and tibia VC were assessed quantitatively on the HR-pQCT images and abdominal aortic VC semi-quantitatively on the lumbar spine QCT images.
Results
In our population, there was no correlation between BMI and the amount of VC at any site. vBMD was higher in obese people compared to normal weight at the radius and tibia ($P=0.0001$), but not the lumbar spine or hip. Lumbar spine vBMD was associated with abdominal aortic VC ($P=0.002$). However, no associations were observed between any other BMD measurement or any other site of VC.
Conclusion
We did not observe the expected association between VC and obesity; this might be due to our exclusion of diabetes. We did observe an association between vBMD and obesity. It is surprising that the well-known inverse association between spine vBMD and VC was only observed at the abdominal aorta, and not at peripheral sites, nor were other vBMD measurements associated with abdominal aorta VC.

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P455

An isoform of fibronectin is responsible for bone loss in hepatic osteodystrophy and its deleterious effects can be prevented by binding the mediating receptor

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Cholestatic liver disease is often associated with increased fracture risk. We had found that circulating levels of a fibronectin isoform called oncofetal fibronectin (oFN) were elevated in patients with primary biliary cirrhosis. Indeed, injecting oFN to mice suppressed osteoblast differentiation and bone mineral density *in vivo*, suggesting it is responsible for bone loss in these patients. The aim of this study was to define the mechanism by which oFN affects osteoblast function and evaluate possible modifiers in experimental hepatic osteodystrophy.
The fibronectin isoform oFN is characterized by the presence of O- and N-glycosylations. Enzymatic O-deglycosylation of this isoform prevented the

inhibition of osteoblast function usually seen with oFN *in vitro*. Either introduction of a mutation at AA 33 of the variable region, or binding of this glycosylated site with a specific antibody normalized osteoblast differentiation. The responsible site binds to α4β1 integrin. Indeed, this integrin α4β1 mediates the inhibitory effect of oFN both *in vitro* as well as *in vivo*. In a hepatic osteodystrophy mouse model, we demonstrate that liver fibrosis is associated with increased circulating oFN and diminished BMD. In addition, trabecular bone loss induced by oFN injection or fibrosis induction was counteracted by either administering an antibody that binds to α4 integrin (PS/2), or the CS1 peptide, which contains a binding site for α4β1 integrin.
In summary, oFN inhibits osteoblast activity through an O-glycosylation in the variable region that results in decreased integrin-mediated signaling. This deleterious effect is prevented by binding α4β1 integrin. Thus, we have characterized a molecule and the receptor mediating bone loss in patients with hepatic osteodystrophy associated with increased oFN, and evaluated possible therapeutic interventions in a murine model.

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P456

Bone mineral density and TBS in patients with mutations of the alkaline phosphatase gene

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Alkaline phosphatase activity is critical for the mineralization of the bone matrix. Indeed, inactivating mutations of the ALPL gene, encoding the isoenzyme expressed in bone and liver, may result in the severe abnormalities of bone and other connective tissues that characterize hypophosphatasia. Nevertheless, the clinical spectrum of hypophosphatasia is rather broad and variable between a within families. Thus, along severe infantile forms, there are adult forms with mild manifestations that may be incidentally discovered by the presence of low alkaline phosphatase activity in serum. Some of those patients may be heterozygote carriers of mutations of the ALPL gene. They may be asymptomatic or have only minor ailments. Yet, it is unclear whether those patients have a normal BMD or not.
In order to clarify this issue, we evaluated spine and hip BMD in 20 individuals with persistently low serum alkaline phosphatase activity (six men and 14 women; mean age 57 years, range 20–77). The results were compared with a group of 80 age- and sex-matched controls.
After sequencing the exons and exon-intron boundaries of the ALPL gene, a mutated allele was found in 10 patients. Patients and controls had the same height and weight. They also had similar BMD at the spine (1.028 ± 0.182 g/cm^2 in patients; and 0.960 ± 0.190 g/cm^2 in controls, $P = 0.15$) and the femoral neck (0.816 ± 0.151 vs 0.785 ± 0.129 g/cm^2). In line with these results, there were no significant differences in the age-adjusted BMD between patients with and without ALPL mutations. The mean trabecular bone score (TBS) was 1.41 ± 0.09; in all cases it was higher than 1.20, the lower limit of the normal range.
In conclusion, this study suggests that patients with heterozygous mutations of the ALPL gene have normal BMD, despite the fact that they have persistently low levels of serum alkaline phosphatase.

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P457

Mutant TGFβ1 in Camurati-Engelamann disease causes systemic manifestations and reproductive disorders more often than previously thought: report of eight Chinese families

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Camurati-Engelmann disease (CED) is a rare autosomal dominant bone disease with transforming growth factor-β1 (*TGFβ1*) gene mutation. In order to expand our limited knowledge of Chinese CED patients, we reported eight Chinese families (11 patients) diagnosed with CED. The study was approved the Department of Scientific Research of Peking Union Medical College Hospital. All the patients were evaluated genetically, clinically, biochemically and radiographically. Changes of clinical and biochemical abnormalities were observed after treatment by prednisone or bisphosphates. Correlations between bone turnover markers and other biochemical parameters were analyzed. Three different heterozygous mutations, R218H, C225R and R218C, were detected in 1 (12.5%), 1 (12.5%) and 6 (75%) families, respectively. Except for some reported common features, such as limb pain and waddling gait, we additionally found that some previously considered uncommon features, especially systemic manifestations, delayed puberty and hypogonadism, had high incidence of 40%–60%, 75% and 62.5%, respectively. Bone turnover marker ALP was significantly and positively associated with inflammatory markers (ESR, $r = 0.945$, $P = 0.001$; hsCRP, $r = 0.867$, $P = 0.012$), while significantly and negatively associated with HGB ($r = -0.692$, $P = 0.027$), which were part of systemic manifestations. Bone pain and all the biochemical abnormalities were relieved dramatically after short-term prednisone treatment, Value of WBC significantly increased after 1-month treatment, meanwhile, values of HGB, PLT, ESR and hsCRP back to normal after 3-month treatment. Our study suggested that CED might be a systemic disorder rather than a purely metabolic bone disease and activated inflammatory responses caused by enhanced TGFβ1 activity might play a crucial role in the pathogenesis of CED. Reproductive disorders were common in CED and might be due to the potential inhibitory role of TGFβ1 in gonad and secondary sex organ development. We recommended short-term corticosteroid as the appropriate therapeutic strategy in CED.

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P458

Colony-stimulating factor 1 receptor a (Csf1ra)-deficient zebrafish as a model of unbalanced bone remodeling

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Osteoclasts are multinucleated giant cells derived from the monocyte/macrophage lineage in the presence of receptor activator of nuclear factor kappa-B ligand (RANKL) and colony-stimulating factor 1 (CSF1). The bone remodeling process in zebrafish is incompletely understood. Here we describe several methods to quantify bone formation and resorption using a zebrafish mutant that lacks functional colony stimulating factor 1a receptor (csf1ra$^{mh5/mh5}$). Mice deficient in CSF1 receptor (CSF1R) are toothless and severely osteopetrotic due to the absence of osteoclasts. Zebrafish have two csf1r genes, csf1ra and csf1rb. It was shown before, that fish lacking csf1ra exhibit deformed neural and hemal arches, and have a decreased number of osteoclasts during fin regeneration.
To quantify osteoclast number and bone resorption, respectively, we stained scales from csf1ra$^{mh5/mh5}$ mutants and WT siblings for tartrate-resistant acid phosphatase (TRAP) and mineralized tissue (Von Kossa). We observed fewer TRAP positive osteoclasts in csf1ra$^{mh5/mh5}$ mutants compared to controls ($P < 0.0001$), which we could also see using a transgenic osteoclast reporter line. Additionally, we found that the area of resorbed bone in the scales of mutant fish was reduced ($P < 0.0001$), confirming the reduction in mature osteoclast numbers in the csf1ra$^{mh5/mh5}$ mutant. Studying the rate of bone formation *in vivo* using calcein and alizarin red staining, revealed that csf1ra$^{mh5/mh5}$ mutants make bone at a higher rate than controls ($P < 0.0001$). To show that, as in mammals, zebrafish develop an osteoclast-poor osteopetrosis phenotype in the absence of a functional Csf1r, we will further analyze bone structure and density by microCT. Here we demonstrate that, as in mammals, zebrafish osteoclastogenesis is dependent on *csf1* signaling. We developed and validated valuable techniques for studying zebrafish mutants having unbalanced bone remodeling and supporting use of forward genetics in zebrafish for identification of enhancers and suppressors of bone remodeling phenotypes.

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P459

Clinical, Biochemical and Radiographic Spectrum of X-linked Hypophosphatemia in Adults

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X-linked hypophosphatemic osteomalacia (XLH) is an inherited skeletal disorder. The pathogenesis of the disease is fibroblast growth factor 23 (FGF23) induced renal phosphate wasting, hypophosphatemia and inappropriate lower 1, 25-dihydroxy-vitamin D_3 concentration, which lead to impaired bone mineralization. There are only a few studies focus on phenotype of XLH patients in adulthood. Therefore, this cross-sectional study systematically described anthropometric, clinical, biochemical and radiological features in adult XLH patients. The study was approved the Department of Scientific Research of Peking Union Medical College Hospital. Adult XLH patients were characterized by short stature (male, 143.7 ± 8.56 cm; female, 140.8 ± 7.55 cm), symptomatic at presentation with skeletal pains, the most frequent sites were knees (34/47, 72.3%), back (27/47, 67.5%), hip (14/47, 29.8%), ankles (11/47, 23.4%), and shoulder (9/46, 19.6%). All subjects had experienced dental problems, 38/46(82.6%) had spontaneous tooth decay, and 35/46 (76.1%) undergo dedentition, while peridental abscess presented in 35/46(76.1%) patients. Almost all the patients had hypophosphatemia (38/42, 90.48%), which were associated with hyperphosphaturia (26.32 ± 16.62 mmol/24h). Half (19/38, 50%) of the patients revealed elevated ALP (127.05 ± 65.05 U/l). Serum parathyroid hormone levels were elevated (93.34 ± 51.71 pg/ml) in 30/42 (71.43%). The radiological spectrum illustrated wide spread distribution of bone deformation, containing lumbar and thoracic vertebra deformation(15/40), flexural deformation of femur (26/40), deformation of pelvis(23/40) and enthesopathy (21/40). Severe and prevalent degenerative joint disease in hip (75%) and knee (62.5%) were observed, and were associated with age and gender. Fracture risk of XLH patients may associate with but not restricted to BMD. Lower sitting height may relate to fracture risk ($P = 0.036$, OR = 0.602). In conclusion, this is the largest study investigating clinical spectrum of adult XLH patients, proposed rarely reported radiologic characters, and analyzed the possible influence factors associated with fracture event.

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P460

Chloride channel voltage-sensitive 7 (CLCN7) loss-of-function zebrafish as a genetic model of osteoclast-rich osteopetrosis

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Osteoclasts are myeloid-derived cells that degrade bone through the localized production of acid and proteases. The catabolic action of osteoclasts is counterbalanced by formation of new matrix by osteoblasts. In mammals, bone resorption and formation are coupled through crosstalk between osteoclasts and osteoblasts. Zebrafish are emerging as a valuable model to study bone biology, but how closely zebrafish osteoclast function parallels that of higher vertebrates is unknown. To determine whether zebrafish osteoclasts utilize the same resorptive machinery as mammals, we generated a zebrafish lacking *clcn7* using the CRISPR-Cas9 technique. In mammals, CLCN7 is required for chloride anion secretion and resorptive function in osteoclasts. Mutations of this protein result in lethal osteoclast-rich osteopetrosis in humans, and a similar phenotype is seen in CLCN7-deficient mice. We find that $clcn7^{-/-}$ zebrafish are viable and fertile. Osteoclast activity was determined by quantification of resorption pits in scales. Osteoclast activity is decreased in the mutant compared with wt fish, when the number and size of resorption pits on the scales are quantified. Micro-computed tomography of mutant fish reveals osteopetrosis and deformity of the vertebral arches, consistent with the observed resorption defects, and indicating that zebrafish also require CLCN7 for normal bone turnover and morphology in zebrafish. Future experiments will address whether osteoclast–osteoblast crosstalk occurs in zebrafish, since patients with *CLCN7* mutations have increased osteoblast activity.

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P461

The role of bone morphogenetic proteins in muscle regeneration of osteoarthritic patients

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Introduction
Age-related bone diseases, such as osteoarthritis (OA) and osteoporosis (OP), are strongly associated with sarcopenia and muscle fiber atrophy. Potential mechanisms involved in the reduction of skeletal muscle mass during sarcopenia converge on the failure of satellite cells in replacing and repairing damaged muscle fibers. Myostatin and bone morphogenetic proteins (BMPs) are molecules able to regulate muscle mass homeostasis by activating satellite stem cells [4]. In this study, we investigated the role of BMP2, BMP4, and myostatin in the pathophysiogenesis of sarcopenia related to osteoporosis and osteoarthritis.
Methods
Muscle atrophy, BMP2, BMP4, and myostatin expression were evaluated in 27 biopsies of osteoarthritic (OA) women and 27 biopsies from osteoporotic (OP) by immunoistochemical reaction. Muscle stem cells niches were investigated by ultrastructural analysis.
Results
We found that OA muscle biopsies showed a significantly higher number both BMP2-positive fibers (62.79 ± 6,205) and BMP4-positive fibers (37.35 ± 5.63) as compared with muscle of OP patients (9.60 ± 1.57 and 13.92 ± 3.343). Unlike BMP2 and BMP4 expression, the number of myostatin-positive fibers in OP patients (33.95 ± 4.10) was significantly higher compared with OA group (13.86 ± 1.68). The ultrastructural analysis of BMPs-positive tissues displayed the presence of a high rate of satellite cells both single or as syncytium giving a proof of muscle regeneration capability.
Discussion
Our results clearly indicated that sarcopenia and osteoporosis shared an impairment of metabolic activity. Conversely, the higher expression of BMPs in OA patients seems to inhibit the onset of age-related sarcopenia. The characterization of molecular mechanisms underlying the bone–muscle crosstalk could open new therapeutics perspectives in elderly diseases.

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P462

Secondary bone size deficit in patients with Ehlers–Danlos syndrome

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Background
Ehlers–Danlos syndrome (EDS) comprises a group of inherited connective tissue disorders, caused by various defects in the biosynthesis or secretion of fibrillar collagens. As collagen represents a major constituent of the bone matrix as well as of tendons and muscle, bone strength in EDS patients might be impaired both via direct and indirect pathways. Although decreased muscle strength, decreased areal bone mineral density (BMD) and increased fracture risk have been reported, no studies have investigated volumetric bone parameters in these patients.
Objective
We aimed to compare volumetric BMD (vBMD) and cortical bone geometry in patients with EDS hypermobility type (EDS-HT) and age- and sex-matched controls.
Methods
Forty-two female EDS-HT patients (mean age 40.0 ± 10.8 years) and 42 controls were included in a cross-sectional study. vBMD and bone geometry at the tibia (4 and 66% region) as well as lower leg muscle cross-sectional area (CSA, 66% region) were measured using pQCT.

Results
Although EDS-HT patients did not differ from controls with regard to trabecular or cortical vBMD, they presented with about 6.3% smaller trabecular bone area ($P=0.014$), 8.9% smaller cortical bone area ($P=0.005$), 6.6% smaller cortical thickness ($P=0.021$), and, albeit non-significant, 2.9% smaller periosteal circumference ($P=0.101$). As a result, strength-strain index was 9.8% lower in EDS-HT patients as compared with controls ($P=0.039$). Furthermore, EDS-HT was associated with a 10.8% decreased muscle CSA ($P=0.004$) without differences in muscle density. Bone/muscle CSA ratio was within the normal range and did not differ between groups.
Conclusions
EDS-HT patients present with both a trabecular and cortical tibial bone size deficit as compared with controls, which might contribute to their increased fracture risk. As indicated by the decreased muscle CSA and normal bone/muscle CSA ratio, this bone size deficit is probably secondary to decreased mechanical loading in these patients with known muscle dysfunction.

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P463

Change in bone mineral density with high-dose prednisone in patients with rheumatoid arthritis

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Background
Recently, we showed that treatment with COBRA-light therapy including prednisone with initially 30 mg/day was as effective as the original COBRA scheme, with initially 60 mg/day (Ter Wee, ARD 2015), in the treatment of rheumatoid arthritis (RA). Since high-dose glucocorticoids are associated with bone loss, we investigated the differences in bone mineral density (BMD) after 1 year of treatment in both arms.
Objective
To determine whether there is a significant difference in BMD between COBRA and COBRA-light, and to determine the change in BMD between baseline and 52 weeks within these groups.
Methods
An open-label, randomised controlled, non-inferiority trial of patients with active early RA following a treat-to-target protocol.
Results
In total, 144 patients were included and randomized to either COBRA ($n=71$) or COBRA-light ($n=73$) therapy. We did not find differences in change in BMD between COBRA and COBRA-light at all sites. However, COBRA-light showed a significant decrease in BMD in the lumbar spine and total hip after 52 weeks as shown in Table 1.
Conclusion
No difference in change in BMD between COBRA and COBRA-light was found. The overall bone loss was small, which suggests that the negative effects of (high-dose) prednisone on bone might be counteracted by the large reduction in disease activity as a result of combination therapy and tight control treatment.

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Table 1 Changes in bone mineral density between baseline and week 52 during COBRA and COBRA-light therapy.

	COBRA ($n=71$)			COBRA-light ($n=73$)		
	Baseline	Week 52	Change	Baseline	Week 52	Change
Lumbar spine	1.12 (0.17)	1.12 (0.17)	0.01%	1.10 (0.15)	1.09 (0.15)	−1.02%*
Total hip	0.95 (0.14)	0.95 (0.14)	0.05%	0.95 (0.12)	0.94 (0.13)	−1.16%*
Femoral neck	0.90 (0.16)	0.89 (0.17)	−0.59%	0.88 (0.12)	0.87 (0.11)	−0.98%*

*Significant change between baseline and week 52 ($P<0.05$). Values are reported as mean (SD), unless otherwise specified.

P464

The course and management of craniofacial fibrous dysplasia: a case series

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Introduction
Cranialfacial fibrous dysplasia (CFD) presents with pain, facial asymmetry and/or neurological complications. It has been suggested that patients with CFD respond favourably to treatment with bisphosphonates, by a decrease in pain and arrest of progression. Therefore, we performed a retrospective study of 56 patients with CFD in our center.
Methods
We assessed clinical characteristics and disease course. Furthermore, clinical and biochemical response on bisphosphonate treatment was assessed. Prognostic factors for treatment outcome were identified.
Results
Most patients had monostotic CFD ($n=36.68\%$), eight had polyostotic FD (14%) and 10 (18%) McCune–Albright syndrome (MAS). Mean age at diagnosis was 23.8 ± 16.1 years and 4.2 ± 4.0 years in MAS, mean follow-up was 16.5 years (range 1–61). Most affected sites were the frontal, sphenoid and maxillary bone, 43, 36 and 32% respectively. Reported symptoms were headaches (26%), visual problems (21.4%), hearing loss (4%), vertigo (4%) and loss of smell (4%). MAS-patients had significantly higher SBS of the skull. In MAS patients GH-excess was diagnosed in 60% solely in patients with a high SBS score of the skull ($P<0.001$) vs patients without GH-excess. In monostotic CFD mean alkaline phosphatase was 114.7 ± 73 U/l at time of diagnosis and 195.3 ± 58 U/l and 630.0 ± 257 U/l in PFD and MAS respectively. Forty-two surgeries were performed in 19 patients (33 surgeries in monostotic CFD, 1 in PFD and 8 in MAS patients), 48% for cosmetic reasons, 14% for pain reduction and 12% for nerve compression. Forty-one patients were treated with bisphosphonate and 96% of the monostotic CFD patients, 75% in the PFD and 20% in MAS had a good response to treatment. High SBS of the skull and GH-excess were risk factors for reduced treatment response.
Conclusion
In patients with CFD, treatment with bisphosphonates reduces pain and clinical symptoms, especially in those with monostotic CFD. GH-excess and high SBS of the skull appear to be risk factors for treatment failure.

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P465

Tracking inflammation in mouse model of fibrodysplasia ossificans progressiva prior to the detection of heterotopic ossification as a potential biomarker

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Fibrodysplasia ossificans progressiva (FOP) is a rare debilitating genetic disease characterized by abnormal progressive heterotopic endochondrial ossification of soft tissues. FOP results from mutations in the intracellular domain of the type I BMP receptor ACVR1 (ALK2) the most common of which is R206H. FOP mutations alter the sensitivity of ACVR1 to Activin A from an antagonist to an agonist. We have previously shown that Activin A is necessary and sufficient for driving heterotopic ossification HO in our mouse model of FOP ($Acvr1^{[R206H]FlEx/+}$; $Rosa\text{-}CreER^{T2}$ mice). HO in FOP patients is often preceded by a flare-up, which might be due to a soft tissue injury or other inflammatory stimuli. Hence detection of inflammation in FOP patients may offer a potential therapeutic window for intervention before HO formation. In $Acvr1^{[R206H]FlEx/+}$; $Rosa\text{-}CreER^{T2}$ mice we visualized the homing of macrophages or phagocytes to the site of injury in response to cardiotoxin-induced muscle injury within 24 h. Subsequently, we observed heterotopic bone formation at the site of injury by day 10. Macrophages and phagocytes that respond to inflammatory stimuli were tracked *in vivo* using inflammation probes that target activated phagocytes with chemiluminescence. Macrophages were also tracked by intravenous administration of radio dense Exitron nano particles, which were taken up by the macrophages enabling monitoring by *in vivo* μCT imaging. Our findings demonstrate that HO formation is preceded by an inflammatory response at the site of cardiotoxin-induced injury. Macrophages and phagocyte that home to the site of injury might be responsible for initiating heterotopic ossification by

secreting agents such as Activin A that drive endochondrial ossification. Further imaging studies using specific and sensitive inflammatory probes aimed at detecting inflammation at the earliest possible time before progressive HO sets in will be valuable in development of new therapeutic interventions.

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P466

Bone histomorphometric alterations and chronic kidney disease in patients with osteoarthritis and osteoporosis

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Osteoarthritis and osteoporosis are two elderly conditions characterized by two different pathophysiological mechanisms. In patients with chronic kidney disease (CKD), there is a metabolic impairment of the muscular-skeletal tissue. The aim of our study is to evaluate the biochemical and morphostructural alterations in different states of CKD in patients with osteoarthritis or hip fracture.
We evaluated all patients undergoing hip replacement surgery from January 2012 to April 2015 for femoral neck fracture or hip osteoarthritis. For each patient, we evaluated bone metabolism and the kidney function by blood chemistry; glomerular filtration was calculated by CKD-EPI method and the patients were divided, according to the degree of CKD, in five groups. For each patient were performed bone mineral density (BMD) evaluation and histomorphometric analysis of the bone sample taken from the femoral head during surgery.
The total of 272 patients (92 men and 180 women, average age 76.1) were included in the study. They underwent surgery for fracture ($n=144$) or osteoarthritis ($n=128$). The patients with grade I ($n=43$) and II ($n=112$) were arthritic respectively in 65 and 60%, while patients with grade III ($n=99$), IV ($n=14$) and V ($n=4$) were fractured respectively 69, 92 and 75%. PTH was lower in patients with grade I and II ($P<0.01$), vitamin D in patients grade III and IV ($P<0.01$). The BMD values were lower with the decrease in renal function both in osteoarthritic patients than in the fractured ones. Histomorphometric analysis showed a progressive decrease of the structural parameters of the bone with a bone volume significantly lower in patients with stage III–IV (0.525 mm^2) compared with grades I–II (0.63 mm^2, $P<0.01$) as well as even a reduced trabecular thickness (13.88 vs 16:54, $P<0.01$).
Biochemical markers could be predictors of bone histomorphometric alterations in patients with CKD in patients with osteoarthritis and osteoporosis.

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Disease activity in patients with rheumatoid arthritis according to vitamin D status

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Introduction
Vitamin D deficiency is common in patients with rheumatoid arthritis and may be related to disease activity.
The aim of the study was to evaluate the association between 25(OH)D level and disease activity in patients with rheumatoid arthritis.
Materials and methods
The study included 61 patients with rheumatoid arthritis, 73.8% were women, 70.4 patients were younger 60 years old. Subjects suffering from liver and kidney insufficiency and those who had received vitamin D in the previous 3 months have been excluded. Disease activity was assessed by DAS-28 score, joint pain degree, morning stiffness time and laboratory measures including Hb and ESR.
The level of $25(OH)D_{total}$ was evaluated by electrochemiluminescence method (Elecsys 2010, Roche). Vitamin D deficiency was defined as a 25(OH)D below 20 ng/ml, and vitamin D insufficiency as 25(OH)D of 21–29 ng/ml.
Results
In patients with rheumatoid arthritis, the frequency of vitamin D insufficiency and deficiency was 32.8 and 55.7% accordingly. 14.7% subjects with rheumatoid arthritis had severe vitamin D deficiency. 25(OH)D was associated with morning stiffness ($r=-0.35$; $P=0.04$), ESR level ($r=-0.36$; $P=0.05$), DAS-28 ($r=-0.24$; $P=0.05$), Hb ($r=0.27$; $P=0.01$).
Summary
Physicians should consider 25(OH)D level in management of patients with rheumatoid arthritis.

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P468

In vitro model of antioxidant prevention of urolithiasis

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Urolithiasis is characterized by formation and retention of solid crystals within the urinary tract. There are numerous causes that may lead to urinary stone formation. However, kidney stones are mostly composed of calcium oxalate that predominantly generates free radicals that are toxic to renal tubular cells.
The aim of the study is to explore the toxic effect of oxalate to renal epithelial cells and to explore possible effects of antioxidants on its inhibition.
Two cell lines were used as *in vitro* model of urolithiasis: Madin Darby canine kidney cells subtype II (MDCKII) and pig kidney epithelial cells (LLC-PK1). Oxidative stress was induced by exposure of cells to sodium oxalate (NaOX) in concentration of 8 mM. In order to prevent oxidative stress, cells were treated with three different concentrations (0.5, 0.1 and 0.05 ng/ml) of L-arginine, an antioxidant. Cytotoxicity of NaOX and the effects of L-arginine were determined by cell counting and light microscopy. The oxidative stress was evaluated by expression of superoxide dismutase (SOD), immunohistochemically (with antiSODI antibody) and by RT-PCR.
In both cell lines treated with NaOX only, cell necrosis was observed. Cell survival in MDCKII cell line was around 60%, while in LLC-PK1 cell line it was significantly less. In both cell lines, cells pretreated with L-arginine prior to NaOX exposure showed lower levels of necrosis, and significantly higher levels of cell survival. Positive correlation of SOD expression was observed in all groups of cells, by immunohistochemistry and by RT-PCR.
Our results indicate that an antioxidant pretreatment with L-arginine of cells later exposed to oxalate toxicity is able to hamper oxalate-induced oxidative stress in kidney epithelial cells and as such could play a role in prevention of urolithiasis. More studies to further evaluate its potential as a prevention agent of urolithiasis are obligatory.

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FGF23 and vitamin D metabolism in chronic kidney disease – mineral bone disorder

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Fibroblast growth factor-23 (FGF23) is a major regulator of phosphate metabolism often elevated in genetic hypophosphataemic disorders and in chronic kidney disease–bone mineral disorder (CKD–BMD). Recent studies have identified relationships between FGF23 and vitamin D.
Objectives
To determine the relationship between vitamin D and FGF23 metabolism in CKD.
Method
We used randomized samples from patients with CKD (eGFR <70) and volunteers from the Ministry of Defense were used as controls. FGF23 concentrations were measured using an enzyme-linked immunosorbent assay for cFGF23 (Biomedica, Vienna, Austria) and for iFGF23 (Immutopics Inc., San Clemente, CA, USA). 25(OH)D (D_2 and D_3) and $24,25(OH)_2D_3$ were measured by LCMS.

Results
cFGF23 concentrations were significantly higher in CKD patient samples (7.2 ± 2.5) than controls (1.47 ± 2.1 pg/ml). Low iron status was observed, 37% of CKD patients showed low iron concentration (13.8 ± 1.8 μmol/l; norm 11.5–30 μmol/l) and a transferrin saturation $<16\%$; 24% showed elevated ferritin with values >300 μg/l. Decreasing eGFR (from 70 to 20) was accompanied by a small decrease in vitamin D status, 25(OH)D from 60 to 40 nmol/l and 24,25(OH)2D3 from 4 to 1 nmol/l; while the ratio of 25(OH)D:24,25(OH)2D3 (mean 23 ± 1) increased.
Conclusion
cFGF23 is raised in patients with CKD as a compensatory response to hyperphosphatemia or phosphate overload. Due to 25(OH)D deficiency, patients with CKD develop secondary hyperparathyroidism which exacerbates bone loss bone disease. 24-hydroxylase, enzyme responsible for the catabolism of both 25(OH)D and 1,25(OH)2D, is rapidly induced by 1,25(OH)2D and FGF-23 and suppressed by parathyroid hormone (PTH). In CKD, net effects of declining renal function and rising FGF23 and PTH concentrations on vitamin D catabolism are not clear. We observed that 24,25(OH)2D3 concentrations are further suppressed in CKD patient with vitamin D deficiency, suggesting metabolism favours the production of biologically active 1,25(OH)2D.

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P470

Dose response radiation induced bone loss: FDG PET-CT shows a threshold effect in changes in metabolism and density

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Radiosurgery of the spine is used to treat patients with spinal metastases. Recent studies have shown increased fracture risk in patients treated with high doses of irradiation. This study tested if there is a dose response relationship between prescribed radiation and induced bone loss and metabolism.
Methods
Vertebral bodies C5–C7 of ~50 week old female Yucatan minipigs were irradiated at 16, 20, and 24Gy ($n=5$/dose). The cervical spine regions were scanned on a clinical PET-CT scanner prior to irradiation, 1 month and 3 months after irradiation. Following CT scanning, dynamic 1h PET scans were acquired after injection with 18Fluorodeoxyglucose (18FDG). CT scans were analyzed using the Mindways' Bone Investigational Toolkit. Changes in volumetric integral and cancellous BMD, mineral mass, and cortical thickness were calculated for the central 75% of the distance between endplates. Pharmacokinetic modeling was done of the 18FDG uptake using Siemens IRW pharmacokinetic software. Statistical analysis of CT and pharmacokinetic data was performed by ANOVA or 2-tailed *t-test* when appropriate.
Results
Cortical thickness and integral BMD increased in a dose dependent fashion. The increase in vertebral mineral mass was greater in the high dose group. Cancellous BMD decreased in a dose dependent fashion. 18FDG retention decreased in the irradiated vertebrae. The overall vertebral metabolic response (ki) showed significant ($P<0.05$) dose response at 3 months between high, medium, and low dose groups. The magnitude of decrease was similar for the 16 and 20Gy doses which was less than the decrease seen in the 24Gy treated group. At 3 months, the flux between the plasma and vertebral bodies (k1/k2) showed statistically significant difference ($P<0.05$) between irradiated and non-irradiated vertebral bodies in the high dose group. Conclusion: In conclusion, radiation decreased bone growth, increased cortical thickness and integral vBMD, and decreased glucose utilization in a dose dependent fashion but with a threshold effect.

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Paediatric bone disease

P471

Bone involvement and intervertebral disc calcifications in beta-thalassemic patients: a retrospective study

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Background
Bone involvement in patients with β-thalassemia is well known, but only few studies have analyzed bone microarchitecture and the prevalence of intervertebral disc calcifications (IDCs) in these patients. The aim of our study was to evaluate the bone quality in a group of patients with β-thalassemia in terms of geometry and microarchitecture properties; moreover, we evaluated the presence of IDCs in these patients.
Material and methods
Our retrospective case–control study was conducted in adults with β-thalassemia (aged 18–50 years). Patients were divided, according with the ISCD, into 2 groups: subjects with BMD Zs ≤ -2.0, below the expected range for age, and subjects with BMD Zs > -2.0, within the expected range for age. Assessment of proximal femur geometry was performed using the Hip Structural Analysis (HSA), providing the following parameters: Hip Axis Length (HAL), Femoral Strength Index (FSI), Cross-Sectional Moment of Inertia (CSMI), Cross-Sectional Area (CSA), Section Modulus (Z), and buckling ratio (BR). Assessment of bone quality was performed using the Trabecular Bone Score (TBS), stratifying subjects into 3 groups: with abnormal (TBS ≤ 1.200), partially altered (TBS >1.200 and <1.350), and normal (TBS $\geqq 1.350$) trabecular microarchitecture. Finally, we evaluated the prevalence of IDCs highlighted by images of Vertebral Fracture Assessment.
Results
We evaluated 49 patients with β-thalassemia, mean aged 35.2 ± 9.6 years, divided into two groups: 25 patients with Zs ≤ -2.0 and 24 patients with Zs > -2.0. There was a statistically significant difference between groups in number of fragility fractures ($P=0.0339$). Furthermore, TBS of patients with Zs ≤ -2.0 was significantly lower than individuals Zs > -2.0 as mean value ($P=0.0006$) and as categorized value ($P=0.0061$). Finally, we evidenced in seven patients (14.29%) the presence of at least one IDC.
Conclusions
Our results showed that β-thalassemia is characterized not only by a reduction in BMD, but also by a geometric and qualitative bone microarchitecture involvement, demonstrated that HSA and TBS should be included in the assessment of these subjects, in order to obtain a proper management and prevention of fragility fractures; furthermore, presence of IDCs might be better investigated.

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Osteogenesis imperfecta: clinical and laboratory aspects, about five cases

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Introduction
Osteogenesis imperfecta is a rare constitutional bone disease. The bone matrix is poor because of the often mutation on the gene coding for collagen I, leading to many fractures. Classification Sillence completed by Glorieux, describes the clinical variability. The management should be done case by case.
Study objective
Evaluate the clinical profile, biological and radiological of 5 newly diagnosed cases.
Material and methods
Descriptive study, conducted in 2015 at the Rheumatology department in collaboration with the orthopedics and pediatric trauma. Inclusion criteria were patients responding to the diagnosis of osteogenesis imperfecta classification. All have benefited from a clinical evaluation of biological explorations, especially calcium phosphate, standard radiological assessments and BMD dual energy X-ray (Hologic). Treatment with pamidronate was considered in all cases, in association with orthopedic and rehabilitative care according indication.
Results
We identified five cases. It was three boys and two girls. The average age was 6.2 years. According to the classification of Silence and Glorious, three cases were authenticated osteogenesis imperfecta type III; iterative fractures, short stature, skeletal deformities with kyphoscoliosis, premature loss of walking

confining patients in wheelchairs, dentinogenesis imperfecta, blue sclera and laxity ligament. Form IV was found in two cases; presence of hypertrophic callus, ossification of the interosseous membrane of the forearm and shortening of the humerus and femur. Paternal history of the disease was found in two patients. Fractures were multiple, predominant in the lower limbs, at the humerus and thorax in one case respectively. The radiological assessment confirmed the fracture lines to members, and spine in two cases. Bone densitometry found severe osteoporosis in all patients, at the three sites. Laboratory tests were normal. Four patients have benefited surgical treatment, using telescopic nails, combined with rehabilitative care. Pamidronate was considered in all patients according to the pediatric protocol.
Discussion and conclusion
Osteogenesis imperfecta is a rare disease, heterogeneous in its presentation. Genes other than type I collagen could be transferred, responsible for a recessive transmission and serious presentation in childhood. A new classification of the disease results in 12 types. Type I is the most benign form, and most common in rheumatology. The management is always multidisciplinary. It is guided by the same considerations as those of other bone fragility; in particular, the compensation of a calcium and vitamin D deficiency. Bisphosphonates remain effective.

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P473

Age of diagnosis of fibrodysplasia ossificans progressiva has a variable onset and a misleading phenotype

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Background
The clinical presentation and the clinical phenotypic characterization and the natural history of Fibrodysplasia Ossificans Progressiva (FOP) is diverse and the natural history of the disease is to certain extent different from one patient to another.
Methods
In a series of eleven patients age range from 0 to 16 years (eight girls and three boys), variable clinical presentations were the landmark of these patients. At birth, all our patients manifested short great toes in a valgus position. Marfan syndrome was the suggested diagnosis in three children aged 3–8 years and in two adult patients. A constellation of deformities such as torticollis, painful spine, and painful and marked limitation of the weight bearing zones were confusing with variable age of onset. Monophalangia associated with Marfanoid habitus, were also a prevailing clinical presentations.
Results
Our results were based up on the appearance of the earliest pathologic feature of FOP in correlation with the clinical presentation. In infants (0–1 year); three infants showed congenital hallux valgus and stiff spine have been encountered. In pediatric group (3–8 years); in this group Marfanoid habitus was the prevailing clinical picture, genetic tests showed no mutation in the *FBN1* gene. Their prime presentation of progressive torticollis with simultaneous development of erythrematous subfascial nodules, most commonly located on the posterior neck and back. In pre and adult group (10–16 years); four patients presented with monophalangia associated with painful movements because of the progressive heterotopic ossification of the spine and the weight bearing zones and marked elevation of alkaline phsophatase. Genetic confirmation has been perfromed in five patients manifested the classical mutation of the *ACVR1* gene. The rest of the patients were assessed via clinical and radiographic phenotepes.
Conclusion
The early recognition of FOP can be performed by noticing the short halluces and thumbs at early infancy and later on the high alkaline phosphatase activity in areas of heterotropic ossification. Misconception of FOP is of common practice and eventually unnecessary diagnostic biopsies might deteriorate the progression of the condition. The detection of *ACVR1* gene mutation was a confirmatory procedure. Interestingly, the timing of the onset and the location of progressive heterotopic ossifications was extremely variable and confusing among our group of patients.

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P474

Defining a new severity classification and searching for a pronostic factor in cherubism: NFATc1 localization is the answer

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Cherubism is a rare genetic disease (OMIM #118400) characterized by a massive jaw bone osteolysis. This pathology appears around 2–5 years old and in the less severe cases spontaneously regresses after puberty. So far the only treatment available is surgery, often disabling and traumatic. As the cherubism pathophysiology is not yet understood, we carried out a thoroughly characterization of the cherubism granulomas from ten unrelated patients to determine the cells involved and find a potential severity marker. Cherubism presents variable phenotype, and as previously described, neither the SH3BP2 mutations nor the epidemiological data can explain the severity we observed in our patients group. However, our work allowed us to demonstrate that the granulomas are heterogeneous both between patients and for the same patient, preventing the definition of any biological marker. But, we were able to redefine the cherubism classification according to the cells and the NFATc1 cellular localization observed on the biopsies, allowing us to propose a better patient management preventing any unnecessary surgery. Ultimately, we confirm tacrolimus as an efficient drug treatment for the more severe cases.

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P475

Validation of a novel scoring system, the radiographic global impression of change (RGI-C) scale, for assessing skeletal manifestations of hypophosphatasia in infants and children

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Hypophosphatasia (HPP) is the rare inherited metabolic disease caused by loss-of-function mutations in the tissue-nonspecific alkaline phosphatase (TNSALP) gene. TNSALP deficiency leads to extracellular excess of inorganic pyrophosphate, a bone mineralization inhibitor. Here, we report the validity and reproducibility of a novel scale to quantify HPP-specific radiographic changes in pediatric patients. The Radiographic Global Impression of Change (RGI-C) is a seven-point scale (-3 = severe worsening, 0 = no change; $+3$ = near/complete healing), designed for comprehensive evaluation of HPP skeletal health. Sequential radiographic studies (chest (<5 years only), knees, wrists) are assessed for improvement or worsening using age-specific hallmarks of HPP developed by expert consensus. Age-specific features include gracile and/or absent bones and chest deformity (patients <5 years), and osteopenia, 'popcorn calcification', and physeal corner defects (patients ≥ 5 years). Features common to both age groups include metadiaphyseal sclerosis, apparent physeal widening, and metaphyseal radiolucencies and/or fraying. Inter-/intra-rater agreements for six raters across three studies were assessed using intraclass correlation defects coefficients (ICC) and weighted kappa coefficients (KC). Concurrent validity was assessed via correlation between RGI-C scores and simultaneous changes from baseline in: Rickets Severity Scale (RSS); Pediatric Outcomes Data Collection Instrument (PODCI) Global Function scale; Child Health Assessment Questionnaire (CHAQ) Disability Index; 6 Minute Walk test (6MWT); and height z-scores (children ≥ 5 years).

Measure	Number of data points	Pearson correlation®	*P* value
RSS	135	−0.664	<0.0001
PODCI	84	0.595	<0.0001
CHAQ	84	−0.589	<0.0001
6MWT	100	0.284	0.0043
Height *Z*-score	108	0.261	0.0065

ICC revealed moderate-to-good inter-rater agreement for patients <5 years (0.65, 227 radiographs; $P<0.0001$) and ≥5 years (0.57, 136 radiographs; $P<0.0001$). Most raters achieved substantial ($n=4$, KC>0.6) or near-perfect ($N=4$, KC>0.8) intra-rater agreement. Linear regression revealed significant correlations with all measured parameters (Table 1). The RGI-C scale is a valid, reproducible measure for assessing clinically important changes in skeletal manifestations of HPP in pediatric patients.

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Childhood fractures in northern Norway: a population-based study, *Fit Futures*

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Background
Fractures are common injuries during childhood. Incidence rates and patterns varies, but population-based data are scarce. The aim of this study was to describe a population based sex, age and maturation specific incidence of fractures at different anatomical sites in a representative sample from regions above the Arctic Circle.
Methods
All fractures in the population based convenient cohort Fit Futures, comprising 1038 adolescents mainly born in 1993–1994, were recorded retrospectively from the local hospital in 2015. We collected details on patient age, sex, and fracture site and merged with data from the directed cohort survey. Radiologist confirmed all fractures.
Results
In a period from birth to cohort scanning, the register presented altogether 316 fractures in 253 individuals, 45% in girls and 55% in boys. The overall annual fracture incidence was 164 per 10 000 persons year under the age of 18 and 173 under the age of 14. Fractures peaked in both girls and boys at a sexual maturation stage corresponding with high growth velocity. The most common site of fracture was the forearm followed by phalanges with 25 and 20% of the fractures respectively. Fracture frequencies were highest in April to June with 32% of all fractures.
Conclusions
The overall incidence of fractures in childhood in Northern Norway corresponds with other reports from Scandinavia. The portion of fractures in girls is higher than in other studies. Both genders seems especially vulnerable in growth spurt during puberty.

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Clinical features and targeted gene sequencing analysis of paediatric hypoparathyroidism

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Objectives
For paediatric patients with hypoparathyroidism, genetic defects should be considered firstly. This study was to investigate the clinical features and analyse gene mutations of Chinese patients with child-onset hypoparathyroidism.
Subjects and methods
We enrolled 35 paediatric patients with hypoparathyroidism at our clinical centre between 1984 and 2014. Clinical characteristics were collected and Targeted next-generation sequencing (NGS) was performed to test ten related gene mutations, including *AIRE, AP2S1, CASR, CLDN16, FAM111A, GATA3, GCM2, PTH, TBCE* and *TRPM6*.
Results
The average age of onset was 8.7 years. There were 33 patients (94.3%) with tetany and 22 patients (62.9%) with seizures at the first visit. Before treatment, the average serum calcium and phosphorus were 1.65±0.34 mmol/l and 2.40±0.53 mmol/l, respectively. And the median PTH concentration was 3.0 pg/ml (range 1.0–9.8). Intracranial calcification was identified in 30 out of 31 patients (96.8%), nine in 11 patients who had made ophthalmologic consultation were diagnosed as cataract. And 11 patients had hypercalciuria. We identified 6 patients (17.1%) with gene defects, including four patients with deletion mutation of *TBX1* and *COMT* gene, one patient with a novel *GATA*3 mutation (c.286delT; p.W96Gfs*99), and a known mutation of *CASR* gene in a family.
Conclusions
The current study suggests that it is necessary to monitoring the complications of hypoparathyroidism, such as hypercalciruia, renal damage and basal ganglia calcification. For paediatric patients, gene testing should be conducted to clarify the aetiology.

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P478

Primary hyperparathyroidism in children and adolescents

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Context
Primary hyperparathyroidism (PHPT) in children is thought to be extremely rare. We reviewed our experience with PHPT to better characterize these patients.
Objective and methods
A total of 62 patients (<18 years) treated in our hospital were respectively analysed to summarize the clinical characterization and molecular genetics in this rare condition in Chinese. The study was approved by the local Ethics Committee of our center.
Results
The mean onset age of the subjects was 14.3±2.8 years with an average duration of course of 3±2.1 years. The female-to-male ratio was 1.3:1. Among them, eight patients(13%) were clinically diagnosed as MEN-1. Fifty-eight patients (94%) were symptomatic with target-organ damage (bone involvement, nephrolithiasis/nephrocalcinosis or acute pancreatitis). Bone involvement, urolithiasis, and acute pancreatitis were presented in 51 cases (82%), 23 cases (37%), and four cases (7%), respectively. Six patients (10%) experienced hypercalcemic crisis. Serum calcium was elevated in all patients with a mean value of 3.14±1.17 mmol/l. The mean value of serum PTH was 865.66±838.70 pg/ml. Sixty patients underwent surgeries. Solitary adenomas, multiple adenomas, hyperplasia and atypical adenoma occurred in 43 (72%), 5 (8%), 9 (15%), and 3 (5%) cases, respectively. No carcinoma was observed. No permanent complication was observed for all patients postoperatively, except for four cases of recurrence. *MEN-1* and *CDC73* mutation analysis were performed in 25 patients. Four patients were found to carry *MEN-1* mutuations and six patients were found to carry *CDC73* mutuations, with a total mutation rate of 40%.
Conclusion
Symptoms of PHPT in children are often more severe and more common than in adults. These patients can be successfully treated surgically like adults. The most common pathology of children with PHPT was adenomas. Mutations of *MEN1* and *CDC73* genes were recommended for a relative high mutation rate.

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Increased bone resorption markers in young patients with inflammatory bowel disease

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Children and adolescents with inflammatory bowel disease (IBD) have defects in bone mineral density (BMD) and bone structure that do not completely normalize with clinical remission.

The objective of the study was to determine bone turnover marker (BTM) concentrations and factors behind altered bone metabolism in a case-control setting.
We measured the bone formation marker PINP and bone resorption markers CTX and TRACP5b in 42 adolescents and young adults (22 females; mean age 18.8 years, range from 10.7 to 25.0) with ulcerative colitis or Crohn's disease and in 42 age and sex-matched control subjects. Study protocol was approved by Research Ethics Committee.
Half of the patients had disease duration over 8.5y and 62% of patients were in clinical remission. Patients with IBD were shorter (mean, 167 vs 173 cm, $P=0.012$) and had lower BMI (20.8 vs 22.4, $P=0.025$) than controls. BMD Z-scores were lower in IBD patients for lumbar spine (mean (95% CI for mean), -0.7 (-1.0 – -0.4) vs -0.2 (-0.5 – 0.1), $P=0.040$) and whole body (-0.5 (-0.8 – -0.2) vs 0.1 (-0.3 – 0.4), $P=0.011$). TRACP5b was higher in patients with IBD (5.6 U/L (4.6–6.7) vs 4.4 (3.9–5.0), $P=0.001$), but no difference in other BTMs was observed, when adjusting for whole-body bone area. In the patient group, all BTMs were significantly lower in postpubertal subjects when compared to prepubertal and pubertal subjects as expected ($P<0.05$). Current use of contraceptive pills associated with lower PINP ($n=6$ vs $n=12$; 39 ng/ml (33–47) vs 94 (73–121), $P<0.001$) and CTX (0.47 ng/ml (0.32–0.69) vs 0.80 (0.61–1.06), $P=0.014$) concentrations in patients with IBD.
In conclusion, bone health of young patients with IBD, though in clinical remission, is compromised. BTMs reflect increased bone resorption in adolescents and young adults with IBD, which could contribute to lower BMD.

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The influence of mobility on bone status in subjects with rett syndrome: a 10-year longitudinal study

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Low bone mass is a frequent complication of subjects with Rett syndrome. It is well known that many factors such as the use of anticonvulsant drugs, the presence of scoliosis, the nutrition status, the low levels of 25OHD and the ambulatory impairment influence the attainment of peak bone mass in Rett subjects. This study aimed to investigate the long-term influences of mobility on bone status in girls with Rett syndrome
In 47 girls with Rett syndrome, serum calcium, bone alkaline phosphatase, 25-hydroxyvitamin D and quantitative ultrasound (QUS) parameters at phalanxes by Bone Profiler-IGEA (amplitude dependent speed of sound: AD-SoS and bone transmission time: BTT) were measured at baseline and after 5 and 10 years. The subjects were divided into two groups: non ambulatory ($n=22$) and ambulatory ($n=25$).
At baseline both AD-SoS and BTT values were lower in non ambulatory with respect to ambulatory subjects, but the difference was not statistically significant. Non ambulatory subjects presented a significantly ($P<0.05$) later onset of age at menarche and lower birth weight with respect to the ambulatory subjects. BMI was significantly lower in non ambulatory subjects than in ambulatory subjects at each time point. At the 5-year follow up both ambulatory and non ambulatory Rett subjects presented a similar reduction in both AD-SoS and BTT. Also at 10-year follow up both non ambulatory and ambulatory subjects showed a significant reduction in AD-SoS (-4.7% $P<0.05$; and -3.4% $P=$NS respectively) and in BTT (-54% $P<0.05$; and -41% $P=0.05$, respectively) with respect to baseline.
In conclusion this longitudinal study suggests that QUS parameters at baseline are markedly decreased in non ambulatory subjects, however, no significant differences were found in the 10-year changes in QUS parameters. Moreover, nutritional status play a key role in the progressive deterioration of bone status in non ambulatory Rett subjects.

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P481

Mutation update and short-term outcome after treatment with active vitamin D_3 in Chinese patients with pseudo-vitamin D-deficiency rickets

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Pseudovitamin D-deficiency rickets (PDDR) is a rare autosomal recessive disorder resulting from a defect in renal 25-hydroxyvitamin D 1α-hydroxylase, which is encoded by the CYP27B1 gene. To our best knowledge, 48 mutations of the CYP27B1 gene have been identified so far. In the present study, we investigated CYP27B1 mutations in seven individuals from six separate families and identified nine different mutations: two novel missense mutations (G194R, R259L), three novel and one reported deletion mutations (c1446delA, c1504delA, c311-321del and c. 48-60del), two novel nonsense mutations (c.85G>T, c.580G>T) and 1 reported insertion mutation (c1325-1332insCCCACCC). Our findings enriched the database of CYP27B1 mutations. To investigate the response to short-term treatment with calcitriol in PDDR patients, we additionally collected clinical data of eight PDDR patients from our previous study and analyzed the changes of clinical manifestations and biochemical parameters after treated with calcitriol in the whole 15 patients. The statistical analysis revealed that serum ALP and PTH significantly decreased after 6-month treatment with calcitriol. The height change of the patients is positively related to the duration of the treatment ($r=0.772$, $P=0.009$), which implicated the importance of long-term calcitriol supplementation for the growth development of children with PDDR.

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Body composition in children and young patients affected by chronic diseases

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We analyzed body composition (DXA, Hologic) in 334 young patients (aged 3–24 years) with chronic diseases, most of them on long-term glucocorticoid (GC) treatment, and monitored its changes over 3–14 years (6.9 ± 6.2 years).
Bone mineral content (BMC), fat mass (FM) and fat-free mass (FFM) were measured on total body (TB), trunk, upper limbs, lower limbs. BMC, FM, FFM were expressed as percentages and compared with age- and sex-matched healthy Italian controls. BMI was also measured.
In 159 patients with nephrotic syndrome, connective tissue diseases, asthma, autoimmune hepatitis, or transplants (aged 3–20 years), GCs had major effects on trunk BMC and FM, related to cumulative dose. Independently of disease and age, BMC decrease and FM increase were higher during the first year of treatment ($P<0.01$ vs baseline), and continued more slowly thereafter.
In 39 walking boys with Duchenne muscular dystrophy (aged 4–15 years), BMC was low for age, and more reduced at lower than upper limbs. At lower limbs, FM progressively increased and FFM decreased over time. The FFM decrease correlated with changes in muscle strength (evaluated by Manual Muscle Testing, MRC scale), $P<0.03$.
In 136 patients affected by cystic fibrosis (aged 3–24 years; 64 F), body composition analysis showed decreased FFM and FM with respect to controls in both sexes, for both TB and the three sub-regions. We observed significant correlations of BMI with FFM ($P<0.01$); FFM with both TB BMC and lower-limbs BMC ($P<0.02$); FFM, FM, and BMI with trunk BMC (FFM showing the highest correlation); FFM with FEV1, a pulmonary function index ($P<0.02$).
In children and young patients, body composition analysis is a powerful tool to evaluate the disease-related deviations from normal; to demonstrate the GC influence on BMC, FM, and FFM; to highlight the relationship between muscular strength/activity and bone.

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Loss of type I collagen telopeptide lysyl hydroxylation causes musculoskeletal abnormalities in a zebrafish model of Bruck syndrome

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Bruck syndrome, a disorder caused by bi-allelic mutations in either *PLOD2* or *FKBP10*, is characterized by flexion contractures and bone fractures and shows strong clinical overlap with the brittle bone disease Osteogenesis Imperfecta. *PLOD2* encodes the Lysyl hydroxylase 2 (LH2) enzyme, which is responsible for the hydroxylation of lysine residues in the type-I collagen telopeptides. This hydroxylation directs cross-linking of the collagen fibrils in the extracellular matrix, which is necessary to provide stability and tensile properties to the collagen fibrils. To further elucidate the function of LH2 in vertebrate skeletal development, we studied a zebrafish model, harboring a homozygous *plod2* nonsense mutation (permit ECD15/68).
Adult *plod2* mutants presented with a shortened body axis and malformed craniofacial structures. μCT scanning showed severe skeletal abnormalities with evidence of bone fragility and fractures. The vertebral column of *plod2* mutants was scoliotic with compressed vertebrae and excessive periosteal bone formation at the vertebral end plates. Furthermore, tissue mineral density (TMD) was shown to be increased in the vertebral centra of the mutants. Near the horizontal myoseptum, the muscle fibers have a reduced diameter and the endomysium, a layer of connective tissue ensheathing the individual muscle fibers, was enlarged in mutant fish. Transmission electron microscopy showed a disturbed organization and altered diameter of type I collagen fibrils in mutant vertebral bone. Reduced telopeptide hydroxylation and cross-linking of type I bone collagen was demonstrated in *plod2* mutants proving the dysfunctionality of lh2 in these mutant fish.
In conclusion, *plod2* mutant zebrafish display reduced type I collagen telopeptide lysyl hydroxylation and cross-linking and disturbed type I collagen fibril formation. The musculoskeletal abnormalities observed in these mutants are concordant with the clinical findings detected in Bruck Syndrome patients. Therefore, the *plod2* zebrafish mutant is a promising model for elucidation of the underlying pathogenetic mechanisms leading to Bruck Syndrome.

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***CRTAP* variants in early-onset osteoporosis and recurrent fractures**

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Early-onset primary osteoporosis is characterized by low bone mineral density (BMD) and increased tendency to fractures in young people. Studies on rare bone diseases, such as osteogenesis imperfecta (OI), have identified several new genes associated with early-onset skeletal fragility. This study aimed to explore the role of variation in the cartilage-associated protein (*CRTAP*) gene in early-onset osteoporosis and/or recurrent fractures. We first used homozygosity mapping and Sanger sequencing to screen the 18 genes known to cause OI in a severely affected 11-year-old Iraqi girl, born to consanguineous parents. A disease-causing novel homozygous stop-gain mutation was identified in exon 1 of the *CRTAP* gene (c.141dupC). The parents of the patient with severe OI, both heterozygous carriers of the *CRTAP* nonsense mutation, were carefully assessed for BMD and spinal morphology but they did not have evidence of skeletal fragility or a carrier phenotype. To determine whether *CRTAP* mutations underlie early-onset osteoporosis and recurrent fractures, we screened the *CRTAP* gene in 96 young subjects with early-onset osteoporosis and/or recurrent fractures using Sanger sequencing. Our analysis did not detect any potential pathogenic variants. In conclusion, we identified a novel duplication in *CRTAP* causing severe OI but we excluded genetic variants in *CRTAP* as a common cause for early-onset osteoporosis and recurrent fractures. In line with this, no evidence for skeletal fragility was observed in the heterozygous carriers of the *CRTAP* stop-gain mutation. These findings suggest that *CRTAP* mutations result only in the previously described OI type III phenotype and do not underlie milder forms of early-onset osteoporosis.

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Preclinical and *ex vivo* imaging

P485

Sex-related differences of morphological and densitometric properties of mandible in silver foxes (*Vulpes vulpes*)

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The aim of this study was to determine morphological and densitometric parameters of mandible obtained from male and female silver foxes (*Vulpes vulpes*). The study was performed on 1-year-old males ($n=7$) and females ($n=8$). Mandible was isolated and subjected to morphological and densitometric analysis using quantitative computed tomography (QCT) method and Somatom Emotion Siemens apparatus (Siemens, Erlangen, Germany) equipped with Somaris/5 VB10B software. Total bone volume (Bvol), volumetric bone mineral density of cortical bone (Cd), mean volumetric bone mineral density (MvBMD) and cortical bone area (CBA) of left and right parts of mandible were determined. The values of Cd and CBA were measured on the same cross-sectional scan of left and right mandible body positioned just after the last molar tooth. Volume evaluation package was used to determine total bone volume (Bvol) and mean volumetric bone mineral density (MvBMD) of each mandible part. Statistical comparison of the investigated parameters of mandible between males and females, as well as between left and right parts of mandible was performed with a use of non-paired Student *t*-test and $P<0.05$ was considered as statistically significant. Total bone volume of mandible was significantly higher in males than in females by 19.6% ($P=0.0003$). Cortical bone area of mandible shown clear tendency to be higher in males (55.83 ± 1.09 mm^2) than in females (51.19 ± 2.06 mm^2; $P=0.067$). Neither MvBMD nor Cd were significantly different between males and females ($P>0.05$).
In conclusion, this study has shown sex-related differences of Bvol and CBA of mandible in one-year-old silver foxes. This study provided also basic data on densitometric and morphological properties of mandible in silver foxes. Elaborated experimental model may serve for further studies (as an alternative for studies on dogs) on bone metabolism regulation in mammals with a use of physiological, environmental, pharmacological, nutritional and toxicological factors.

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P486

Age-related changes in 3D bone microstructure are more pronounced in the sub-endplate region than in the central region of human vertebral bodies

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The vertebral body microstructure of young individuals appears to be divided into three equally high horizontal regions: two adjacent to the endplates and one in the centre of the vertebral body. With age this subdivision of the vertebral microstructure seems to vanish. The aim of the study was to investigate the differences in the age-related changes in vertebral microstructure in the two regions.
Vertebral (L2) bone specimens from 41 women and 39 men aged 18–96 years with an even age-distribution were μCT scanned. The bone specimens were divided into two regions: one spanning the central third and another spanning the two remaining thirds using custom made software. Standard 3D microstructural parameters were determined in each of the two regions.

In both regions, BV/TV, Tb.N, and vBMD decreased significantly with age, SMI, Tb.Sp, and bone material density increased significantly with age, while Tb.Th was independent of age. In the central region connectivity density (CD) and the degree of anisotropy (DA) were independent of age, while in the sub-endplate region these two parameters decreased and increased significantly with age, respectively. The slope of the fit lines was significantly larger for CD, Tb.N, and DA in the sub-endplate region than in the central region. The age-related changes in CD, Tb.N, and DA differed significantly in the two regions.
The bone in the sub-endplate region was denser, more well-connected, and less mineralised, than the bone in the central region, and the trabeculae in the sub-endplate region was more closely spaced, more rod-like, and of similar thickness as in the central region. Finally, the 3D bone microstructural parameters change significantly differently with age in the two regions. Therefore, caution should be exercised when examining the age-related changes of the 3D bone microstructure of human vertebral bone.

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P487

The CAM assay for human bone regeneration evaluation: the potential of Laponite® clay gel for growth factor delivery *ex vivo*

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An increasing number of biomaterials are in development, seeking to mimic the natural cascade of events during fracture repair. However, these biomaterials need to be rigorously tested prior to clinical application. *In vitro* testing lacks the physiological environment, while *in vivo* studies do not always predict the patient response. Here we hypothesize that the chorioallantoic membrane (CAM) assay can be used to culture human living bone and we aim to examine the potential of this system to test biomaterials. Specifically, we examined the addition of a novel clay hydrogel, Laponite®, as a vehicle for growth factors to enhance bone formation. Empty-cored bone cylinders were extracted from freshly isolated human femoral heads and perfused with Laponite®, Laponite®-VEGF, Laponite®-BMP2 or Blank before culture over 7 days *in vitro* or on Green fluorescent protein-labelled chicks (GFP-CAM). Micro computed tomography (μCT) was conducted on the cylinders to quantify the change of bone volume, followed by histological examination. Histological analysis demonstrated the invasion of chick vasculature into the human tissue evidenced by the presence of avian capillaries in the bone marrow space. Immunohistochemical detection of GFP, collagen type II and Sox9 showed the presence of newly deposited collagenous matrix and cell condensations, which co-localized with avian cells (GFP+) on the CAM-implanted cylinders. μCT indicated a significant increase in bone volume compared to *in vitro* and control groups ($P<0.001$). Critically, addition of Laponite®-BMP2 resulted in the highest increase in bone volume (7.84% ± 5.2 S.D.), followed by Laponite®-VEGF (7.84% ± 5.4 S.D.), compared to Laponite® (3.99% ± 5.0 S.D.). These studies demonstrate the potential of the CAM to integrate human bone tissue and provide a surrogate blood supply to aid regeneration. This avian-human system in combination with the histological and μCT analysis provides a simple alternative preclinical model for the screening of novel biomaterials in bone tissue engineering.

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P488

Mandibular bone: an unusual trabecular bone?

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Introduction
Mandibular alveolar bone appears to be particularly sensitive to local factors compared to other skeletal sites. In growing rats, occlusal hypofunction leads to a dramatic decrease of the alveolar bone microarchitecture. Intra-radicular bone surrounding teeth is a specific functional area with a high bone turnover. We hypothesized that the mechanical loading of the alveolar process during mastication may play a role in the preservation of the alveolar bone microarchitecture. The aim of this study was to compare mandibular bone with tibial bone microarchitecture in adult rats.
Material and methods
Nine 6-month-old female Sprague-Dawley rats (Janvier Lab, Laval, France) were used in this study as approved by the local animal ethics committee. MicroCT *ex vivo* analyses were performed with a Skyscan 1172 (Bruker, Kontich, Belgium). Classical bone parameters were assessed on the central region of the mandibular condyle, the interradicular alveolar bone of the first molar, and the secondary spongiosa in the proximal tibia.
Results
Trabecular bone volume increases respectively in the alveolar process (+44%, $P<0.001$) and in the condyle (+58%, $P<0.001$) compared to tibia. Trabecular number and thickness increase in both alveolar (respectively +15%, +38%, $P<0.001$) and condylar bone (respectively +22%, +50%, $P<0.001$) compared to tibia. On the other hand, trabecular separation decreases in alveolar and condylar bone (respectively −5%, −50%, $P<0.001$). Interestingly, bone marrow volume decreases in the alveolar (−36%, $P<0.001$) and in the condylar bone (−63%, $P<0.001$) compared to tibia.
Conclusion
Despite the fact that tibia and mandible are both submitted to mechanical loading, mandibular bone shows a more dense trabecular network than tibia. Present data suggest that mandibular bone is a unique skeletal-site submitted to specific regulations. Due to its particularly high trabecular density, it would be of interest to determine its response to usual pathological context such as osteoporosis, especially estrogen-deprivation in rats.

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P489

Quantitative assessment of radial bone structural distribution in the proximity of degradable implants by micro-computed tomography

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Degradable bone implants may provide improved long-term healing, tissue remodeling and quality of life. In order to optimize alloy composition and degradation properties, *in-vivo* monitoring of the degradation process and its impact on bone formation is essential because degradation progresses differently *in-vivo* versus *in-vitro*. We aim to understand how degradation of magnesium alloy implants influences bone remodeling and bone structure using Micro-Computed Tomography (μCT).
Mini-screws composed of three different alloys (Mg2Ag, Mg10Gd, WE43 and titanium) have been implanted into rat femurs. After 3 month the complete femur samples have been excised. μCT scans have been performed (Scanco VivaCT 80, 70 kVp, 114 mAs, 1500 projections/180°, 15.4 μm isotropic voxel size, beam hardening reduction kernel, bone mineral density calibration). For the samples of low degradation, a layering approach has been used to investigate the lamellar changes perpendicular to the implant surface, for those in a further progressed degradation stage, density histograms restricted to volumes of interest around the initial (guessed) implant position were evaluated.
Compared to commonly used Ti-implants, imaging of Mg-alloys in μCT is less affected by metal artefacts and extraction of meaningful density profiles is feasible using μCT. In the scenario of strong degradation and non-uniform progression, the separation of bone and implant material remains a challenging task. A time-lapse *in-vivo* μCT study can elucidate the progression of the degradation, especially where corrosion is not taking place exclusively at the surface. Because the characterization of the bone-implant interface is very important for the understanding and refinement of degradable implant materials due to its influence on implant fixation, ultra-high resolution imaging should be combined with our μCT data in a hierarchical approach. Also multimodal approaches (spectroscopy, molecular imaging, histology and others) will help to better understand the complex processes involved in bone healing in the presence of degradable implants.

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Steroid hormones and receptors

P490

Phosphorylation of S122 in ERα is important for the skeletal response to estrogen treatment

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It is well established that estrogen, mainly via estrogen receptor alpha (ERα), has positive effects on bone, but estrogen is not considered as a treatment option against osteoporosis due to negative side-effects in other tissues. ERα is widely subjected to posttranslational modifications (PTMs), which can affect cellular responses to estrogen in a tissue specific manner by influencing the function of ERα and its interactions with other proteins. The *in vivo* role of PTMs of ERα for the skeleton is unknown but the PTM site S122 in ERα is known to modulate ERα transcriptional activity *in vitro*. Our aim was to investigate if phosphorylation of the PTM site S122 in ERα is involved in ERα-mediated bone effects *in vivo*. To this end, we used mice with a point mutation in S122 (S122A) and compared them to WT littermates. Twelve-week-old mice were ovariectomized and treated with estradiol (E_2, 160 ng/day per mouse) or vehicle for four weeks. Tibiae were analyzed using μCT and humeri were exposed to a three-point bending test. E_2 treatment increased cortical thickness in both WT and S122A females, however, the cortical thickness in E_2-treated S122A mice was significantly decreased compared to E_2-treated WT mice (-6%, $P<0.05$). Importantly, the functional three-point bending test demonstrated that E2 treatment increased maximal load at failure (Fmax) in WT mice ($+25\%$ $P<0.001$) while no significant effect of E_2 treatment was observed in S122A mice, demonstrating that phosphorylation of S122 is crucial for the E_2 effect on mechanical strength of cortical bone. In conclusion, our results show that the S122 phosphorylation site is involved in the skeletal response to estrogen treatment and this finding is the first *in vivo* proof of a physiological role of phosphorylation in ERα.

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P491

Exposure to chronic stress induces bone loss via glucocorticoid signalling in osteoblasts

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Chronic stress and depression are associated with alterations in the hypothalamic-pituitary-adrenal signalling cascade and considered a risk factor for bone loss and fractures. However, the mechanisms underlying the association between stress and poor bone health are unclear. Utilising a transgenic (tg) mouse model in which glucocorticoid signalling is selectively disrupted in mature osteoblasts and osteocytes ($HSD2^{OB}$-tg mice), the current study examines the impact of chronic stress on skeletal metabolism and structure.

Eight-week-old male and female transgenic mice and their WT littermates were exposed to chronic mild stress for the duration of 4 weeks. Stressors included restraining, exposure to hot and cold, tilted cages and overnight illuminations. At endpoint, L3-vertebrae and tibiae were analysed by micro-CT and histomorphometry, blood was collected for markers of bone turnover.

Compared to the non-stressed controls, exposure to chronic stress resulted in loss of vertebral trabecular bone mass in male WT mice but not in $HSD2^{OB}$-tg male littermates (WT: -15.9% tg: $+2.8\%$, $P<0.05$). Bone loss in mice with intact osteoblastic glucocorticoid signaling was due to a decrease in trabecular number (WT: -14.3% tg: $+0.8\%$, $P<0.01$) and an increase in trabecular separation (WT: $+12.1\%$ tg: $+1.2\%$, $P<0.05$). While trabecular bone in the tibia was unaffected in stress-exposed WT and $HSD2^{OB}$-tg males, tibial cortical area (WT: -11.1% tg: $+1.3\%$, $P<0.05$) as well as cortical area fraction (WT: -9.5% tg: -2.6%, $P=0.054$) were reduced in stressed WT but not in stressed $HSD2^{OB}$-tg male mice. Histomorphometry and measurements of serum TRAP5b revealed an increase in osteoclast activity in WT males following stress exposure, an effect that was absent in $HSD2^{OB}$-tg males. Interestingly, in female mice, both vertebral and long bone structural parameters remained unaffected by chronic mild stress.

We conclude that in male mice, bone loss during chronic mild stress is mediated via glucocorticoid signalling in osteoblasts and subsequent activation of osteoclasts.

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P492

Distinct glucocorticoid receptor functions during inflammation and osteoporosis

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Glucocorticoids (GCs) are widely used to treat acute and chronic inflammatory diseases such as acute lung injury (ALI) and rheumatoid arthritis (RA) and lead to multiple side effects including GC induced osteoporosis (GIO). Molecularly GCs act by binding to the GC receptor (GR), a ligand induced transcription factor. We discovered that pharmacologically GC exposure reprograms binding of GR monomers towards GR dimers (Genome Research 2015 25:836). Furthermore we challenged the dogma that transrepression by GR monomer suffices for anti-inflammatory activities of GCs, whereas GR dimers would cause side effects such as osteoporosis. We revealed in transgenic mice that transrepression of genes by the GR is not sufficient to suppress inflammation in mouse models of arthritis (PNAS 2011 108:19317) and acute lung injury (Nat Comm 2015 6:7796). Surprisingly, synergistic activity of the GR in concert with pro-inflammatory signaling is required for the induction of anti-inflammatory acting genes.

In contrast GC induced osteoporosis (GIO), the most secondary osteoporosis and a major side effect of steroid therapy, depends on GR mediated transrepression of genes mainly in osteoblasts (Cell Metabolism 2010 11:517).

By setting up an siRNA screen in pre-osteoblasts we functionally characterized novel GR target genes involved in osteoblast differentiation, which could serve as novel drug targets to avoid GIO.

Our work defines new criteria for novel GR modifying compounds and provides new GR target genes that can be addressed to optimize anti-inflammatory therapy by avoiding deleterious effects on bone.

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Late Breaking Abstracts

LB1

All-trans retinoic acid can antagonize osteoblastogenesis induced by different BMPs irrespective of their dimerization types and dose- efficiencies

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Introduction
Alcoholism can result in a compromised regenerative capacity of bone and delayed osteointegration of dental implants. One of the mechanisms is that the thereby overdosed all-trans retinoic acid (ATRA), a main metabolite of alcohol, can significantly inhibit osteoblastogenesis. Bone morphogenetic proteins, potent osteinductive growth factors, can be applied to promote osteogenesis. We previously showed that heterodimerized BMP2/7 could promote osteoblastogenesis in a significantly higher dose-efficiency than homodimerized BMP2 or BMP7. In this study, we wish to uncover the antagonism of ATRA to the BMPs of different dimerization types and dose-efficiencies.
Materials and methods
We assessed the antagonism of 1 μM ATRA to either heterodimerized BMP2/7 or homodimerized BMP2 or BMP7 at 50 ng/ml in a well-established eosteoblastogenesis model with pre-osteoblasts (MC3T3-E1). We measured the following parameters: metabolic activity, alkaline phosphatase activity (early differentiation), osteocalcin (late differentiation), mineralization (final differentiation) and the expression of osteoblastogenesis-related genes.
Results
All the three BMPs could significantly enhance ALP, osteocalcin expression and osteoblastogenesis-related genes and BMP2/7 exhibited a significantly higher efficiency than BMP2 or BMP7. ATRA could significantly antagonize such effects of both heterodimerized BMP2/7 and homodimerized BMP2 or BMP7. On the 28th day, BMP2/7, BMP2 and BMP7 resulted in 3-, 1.79- and 1.24-fold mineralization respectively compared with the control (no BMP, no ATRA). However, the addition of ATRA led to a significantly decreased mineralization to a similar level as the control irrespective of BMPs' dimerization types and potencies.
Conclusions
The advantageous osteoinductivity of heterodimerized BMP2/7 over the homodimerized ones was significantly compromised by ATRA.

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LB2

Rat femoral neck research: an alternative method of histological sections

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Histomorphometry is often adopted as a methodological approach in research of femoral bone structure. However, it is common do not describe technical details of procedure especially on the steps of macroscopy, paraffin embedding and microtomy. So we propose a simplified, reliable and reproducible method of histological processing for intertrochanteric region and femoral neck of mice, to ensure the achievement of tissue sections with similar structures, cell populations and histometric dimensions. Using a board-guide, femurs of 20 animals (ten males and ten females) aged 120 days were crosswise sectioned in the metaphysis, exactly 2 mm below the line tangent to the lower limit of the femoral head. Standard histological sections were obtained in four equidistant levels (1 mm) involving the plans of: a) the base of the intertrochanteric region; b) intermediate the intertrochanteric region; c) base of the femoral neck (BFN); and d) intermediate region of the femoral neck. CBF was measured by histometry in total bone area, medullary and cortical area, total bone diameter, cancellous bone diameter, and cortical width. Statistically the numerical results demonstrated effectiveness in achieving similar histological sections, with the advantage to view the entire outer edge of the bone tissue and obtain cuts in different tissue levels. In conclusion, this alternative methodology can contribute for the study of bone tissue, ensuring the reproduction of the samples data and the reliability of the histomorphometric results of a research.

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LB3

Peripheral quantitative computed tomography measures contribute to the understanding of bone fragility in low-trauma fracture patients

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Background and aims
Dual energy X-ray absorptiometry (DXA) as currently utilised has limitations in identifying patients with osteoporosis and predicting fractures, since most low-trauma fracture (LTF) patients have osteopenia not osteoporosis based on DXA assessment. We aimed to express peripheral quantitative computed tomography (pQCT) variables of patients with low-trauma fracture as *T*-scores by using *T*-score scales obtained from healthy young women, and to evaluate the potential clinical utility of pQCT to complement DXA for the assessment of bone fragility.
Methods
Fracture patients were recruited from a fracture liaison service at a tertiary hospital. Reference pQCT data were obtained from studies of women's health conducted by our group. A study visit was arranged with fracture patients, during which DXA and pQCT measures were obtained to assess their bone strength.
Results
A total of 59 fracture patients were recruited, and reference data were obtained from 78 healthy 19–25 year-old females after screening for medical exclusions. All DXA variables and most pQCT variables were significantly different between healthy young females and fracture patients ($P<0.05$), except polar stress strain index (SSIp; $P=0.15$). Fracture patients were divided into osteoporosis and non-osteoporosis groups according to their DXA *T*-scores. Significant differences between these groups were observed in most pQCT variables ($P<0.05$), except trabecular area and cortical density ($P>0.9$ and $P=0.5$, respectively). By applying pQCT T-scores, 15 (37%) LTF patients who were classified as low/medium risk of fracture on DXA *T*-scores alone were reclassified as high risk. Results of logistic regression suggested trabecular volumetric BMD and SSIp were independent predictors of fracture risk status.
Conclusions
More patients can be identified as having high fracture risk by applying pQCT *T*-score variables in older people with low-trauma fracture. Peripheral QCT *T*-scores contribute to the understanding of bone fragility in this population.

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LB4

Bone mineral density in anorexia nervosa patients

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Anorexia nervosa (AN) is an eating disorder characterized by severe undernutrition. Osteopenia is a frequent, often persistent complication of anorexia nervosa (AN) in adolescent girls and occurs during a critical time of bone development. To determine the impact of chronic undernutrition on bone mineral accrual we measured bone mineral density by dual energy x-ray absorptiometry, bone metabolism markers and leptin levels in AN patients ($n=19$, age 22.67 ± 1.9 years, BMI 14.13 ± 0.39 kg/m^2), with persisting amenorrhea in contrast to age matched healthy female controls ($n=10$, age 23.33 ± 3.01 years, BMI 20.10 ± 0.87 kg/m^2).
We assessed: bone markers (serum osteocalcin, C-telopeptide of type I collagen -sCTX, vitamin D and PTH), and serum leptin levels measured by Linco Kit radioimmunoassay. BMD was measured by DXA at the lumbar spine. Data were presented as mean ± S.E.M.
We showed that there was a significant change in AN patients to healthy controls in vitamin D levels (38.62 ± 6.81 vs 77.50 ± 13.61 nmol/l, $P<0.01$), leptin levels

(4.1±0.49 vs 9.7±2.1 ng/ml, $P<0.01$) and z-score (−2.56±1.2 vs 0.67±0.2, $P<0.01$).
In both groups we found positive correlation between leptin and osteocalcin (Pearsons $P<0.05$), leptin and z-score ($P<0.08$), but negative correlation between leptin and CTx ($P<0.06$), and leptin and vitamin D levels ($P<0.05$), In AN patients, osteocalcin positively correlated with PTH (Pearsons, $r=0.667$, $P<0.05$), and with vitamin D ($r=0.717$, $P<0.03$), but negatively with t-score ($r=-1.00$, $P=0.00$).
Our findings confirmed deleterious effects of undernutrition and on bone metabolism and failure to achieve peak bone mass in AN patients. Consequences of low body weight in AN are hipoleptinemia, which is imbalance of bone turnover (high bone resorption and low bone formation), associated with low BMD.

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LB5

Posterior migration of fusion cages in degenerative lumbar disease treated with anterior lumbar interbody fusion

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Introduction
Most reports focused on clinical advantages of intervertebral cages; only a few studies reviewed the complications. As one of the major complications, cage migrating into vertebral body or spinal canal may result in disastrous consequence. Multiple risk factors may result in cage migration, such as geometric design of cage, surgical technique, the bone quality and post-operative protection. The following is a presentation of nine patients with migrated cage.
Materials and methods
From 2010 to 2015, there were total nine cases of cage migrations in our institution. Posterior migration in seven cases with dura compression, anterior migration in one case, and subsidence of inferior endplate in one case. Seven cases treated with anterior approach, and two cases treated with posterior approach.
Results
Successfully removal of cage was performed in eight cases, and acceptable image outcomes in seven patients. Repeated cage subsidence occurred in one case due to new cage over sizing and limited distraction of previous posterior instrumentation. Failed in cage removal through anterior approach in one case due to great vessel adhesion. After revision or removal surgery, residual numbness or sciatica was noted in three patients. Six cases were with satisfactory or acceptable outcome.
Discussion
Spine fusion is not always an effective treatment for chronic low back pain. Complications such as cage migration may result in severe sequelae, and revision surgery is technically demanding. The anterior approach is a reliable technique for removal of migrated cages.

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LB6

Sclerostin blockade and zoledronic acid improve bone mass and strength in mice with exogenous hyperthyroidism

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Hyperthyroidism in mice is associated with a low bone mass, an increased bone turnover and high serum levels of sclerostin, a potent Wnt inhibitor. Here, we explored the effects of either reducing bone turnover with bisphosphonates or increasing bone formation with neutralizing sclerostin antibodies (Scl-Ab) on bone mass and estimated strength in hyperthyroid mice.
Twelve-week-old C57BL/6 male mice were rendered hyperthyroid by adding L-thyroxine (T_4) to the drinking water (1.2 µg/ml). Saline, 20 mg/kg Scl-Ab 2×/weekly or 100 µg/kg zoledronic acid (ZOL) 1×/weekly were administered to hyperthyroid and control mice for 4 weeks.
MicroCT analysis revealed a lower trabecular bone volume at the spine (−27%) and the distal femur (−48%) in hyperthyroid mice compared to euthyroid controls. Scl-Ab treatment of hyperthyroid mice increased the trabecular bone volume at the femur 2.5-fold as compared to PBS-treated hyperthyroid mice and ZOL twofold. Similar trends were seen for the spine. Cortical thickness at the femoral diaphysis was lower in hyperthyroid mice and increased through both treatments, with Scl-Ab having a more potent effect than ZOL (+12%, $P<0.05$). Bone stiffness estimated using finite element modeling at the lumbar vertebra was 49% lower in hyperthyroid mice and was increased three- and twofold after Scl-Ab and ZOL treatment, respectively. Levels of P1NP were 2.7-fold higher in hyperthyroid mice than in euthyroid controls, and treatment of hyperthyroid mice with Scl-Ab led to a further 13% increase in P1NP, whereas ZOL reduced P1NP concentrations by 23%. CTX levels were likewise 47% higher in hyperthyroid mice as opposed to euthyroid controls, remained unchanged after Scl-Ab treatment and decreased after ZOL treatment (−23%).
Thus, both, anti-resorptive and bone-forming treatments are effective in preventing bone loss in hyperthyroid mice, yet via different mechanisms, and may also be useful for the treatment of patients with hyperthyroidism-induced bone disease.

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LB7

Dose bisphosphonate-based anti-osteoporosis medication affect osteoporotic spinal fracture healing?

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Introduction
The purpose of this prospective study is to investigate whether bisphosphonate-based anti-osteoporosis medication affects fracture healing and clinical outcomes of conservatively treated osteoporotic spinal fractures (OSFs).
Method
A total of 105 patients who were diagnosed with acute OSFs were prospectively enrolled. According to their previous medication history, the patients were allocated into group I ($n=39$, no history of bisphosphonate use) or group II ($n=66$, history of bisphosphonate use). Clinical outcomes were assessed using visual analogue scale (VAS), and Oswestry disability index (ODI). Radiographic parameters including changes in height loss and kyphotic angle at the index vertebra were measured, and radiographic findings suggesting impaired fracture healing such as intravertebral cleft (IVC) sign and fracture instability were evaluated. Univariate and multivariate regression analysis were used to identify related factors.
Results
There were no significant differences in the last VAS and ODI between groups. There were also no significant differences in the radiographic parameters. Although the IVC sign was seen more commonly in group II (30.3%) than in group I (20.5%), fracture instability combined with IVC was noted in the same number of cases. On multivariate regression analysis, medication history showed no significant relationship with the clinical parameters. However, the presence of the IVC sign was related to medication history (odds ratio 4.8; 95% CI 1.01–22.69).
Conclusions
Bisphosphonate use dose not significantly affect the clinical results during conservative treatment for OSFs. However, the occurrence of the IVC sign was related to medication history. Although further studies are needed to verify our findings, there results suggest that suspension of bisphosphonate use should be considered during the fracture healing period for acute OSFs.
Keywords: Bisphosphonate, non-union, osteoporosis, osteoporotic spinal fractures.

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LB8

Bone texture modifications during bone regeneration and osteocyte cell-signaling changes in response to treatment with Teriparatide

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Teriparatide is the active fragment (1–34) of the endogenous human parathyroid hormone (PTH). Studies showed that chronic administration of PTH results in decreased bone mass while intermittent exposure to PTH activates osteoblast bone deposition. Most of the structural studies published so far about the effect of Teriparatide focused their attention on the amount of newly-deposed bone

without investigating the quality of the newly-formed bone tissue; moreover, most of the papers enhance the attention to the osteoblast's cellular and molecular involvement during the process of bone repair, ignoring the pivotal role that osteocytes play in the bone environment by means of the modulation of their signaling. During bone regeneration, after a preliminary formation of fibrous tissue with massive vascular proliferation, two different types of osteogenesis follow each other: firstly the process of Static Osteogenesis, which produces preliminary bony trabeculae with woven texture and, later, the process of Dynamic Osteogenesis, in which osteoblasts, driven by osteocytes, produce a more ordered and more mechanical valid bone. This work includes both *in vivo* and *in vitro* investigations: i) *in vivo* morphological analyses of qualitative repair of experimentally induced lesions in diaphysis of rats femurs and ii) *in vitro* study of the osteocyte cell-signaling, both in response to Teriparatide treatment, in order to deepen the knowledge of morphological and molecular events occurring in bone regeneration, with particular attention to the osteocyte signaling. The results obtained showed that the intermittent administration of Teriparatide *in vivo* anticipates the beginning of Dynamic Osteogenesis, which is characterized by the production of a more ordered and resistant bone. The molecular biology data, performed on an osteocyte immortalized cellular model (MLO-Y4) treated *in vitro* with the drug at different time points showed, by microarray and western blot analysis, different gene/protein expression involved in osteocyte signaling compared with the control condition.

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LB9

First X-linked form of osteogenesis imperfecta, caused by mutations in MBTPS2, demonstrates a fundamental role for regulated intramembrane proteolysis in normal bone formation

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Osteogenesis imperfecta (OI) is a heritable bone dysplasia with collagen-related defects. Dominantly inherited OI is caused by structural defects in type I collagen or IFITM5, while recessive forms are caused by deficiency of proteins that interact with collagen for modification, folding or cross-linking. We have identified the first X-linked form of OI, caused by a defect in regulated intramembrane proteolysis (RIP). One type of RIP involves sequential cleavage of regulatory proteins, transported from the ER in times of stress or decreased sterol metabolites, by site-1 (S1P) and site-2 (S2P) proteases, releasing N-terminal fragments that activate gene transcription.
In two pedigrees with moderately severe OI, linkage analysis and next generation sequencing identified novel mutations in *MBTPS2* (S2P), p.N459S and p.L505F, respectively, located in or near the motif required for metal ion coordination. Neither *MBTPS2* transcripts nor protein stability were decreased. Mutant cells and reporter constructs demonstrated impaired cleavage or activation of RIP substrates OASIS, ATF6 and SREBP. Fibroblasts from X-OI probands have significantly reduced type I collagen secretion, consistent with impaired OASIS signaling. Furthermore, extracellular matrix deposited by cultured proband cells has a decreased proportion of collagen with mature crosslinks, suggesting that impaired collagen crosslinking might undermine bone strength in X-OI. A proband bone sample, which became available after the regular abstract submission deadline, contained type I collagen with less than half the normal level of hydroxylation of Lysine 87 (K87), the residue crucial for intermolecular crosslinking. This finding is consistent with decreased Lysyl Hydroxylase 1 levels in proband osteoblast lysates and increased proband urinary LP/HP crosslink ratios. Proband cultured osteoblasts have broadly defective differentiation, with impaired expression of transcripts related to osteoblast maturation and RIP pathways, such as *ALPL*, *CREB3L1* (OASIS), and *SMAD4*. These studies demonstrate that RIP pathways play a fundamental role in bone development, in addition to their role in cholesterol metabolism.

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LB10

Abstract unavailable.

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LB11

25(OH)D_3 half-life is not influenced by vitamin D supplementation dose

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There is wide variation in the dose-response to oral vitamin D and the increment in plasma 25(OH)D decreases per unit vitamin D given. We hypothesised that this is related to increased 25(OH)D catabolism as reflected in 25OHD half-life (25(OH)D_3 $t_{1/2}$).
Design
25(OH)D_3 $t_{1/2}$ was measured with a stable isotope (SI) technique in older (70y+) men and women in the UK during winter before ($n=47$; 0kIU) or after 9–10 months of supplementation ($n=20$, 24, 22 for 12k, 24k or 48k IU Vitamin D_3/month, respectively). Plasma concentrations of the SI of 25(OH)D, 25(OH)D and 24,25$(OH)_2$D were measured by LC-MS-MS and 1,25$(OH)_2$D by RIA.
Results
Plasma 25(OH)D_3 $t_{1/2}$ did not significantly differ before and after supplementation ($P=0.35$) and between groups with no apparent dose-response (ANOVA for group difference $P=0.05$); mean(S.E.M.) was 20.3(0.45), 22.6(1.1), 20.6 (0.65) and 19.8(0.58) days for 0, 12, 24 and 48kIU, respectively. Vitamin D supplementation was associated with a significantly higher plasma 25(OH)D [49(3.7), 73(5.2), 77(3.1) and 97(4.7) nmol/l; $P<0.0001$] and 24,25$(OH)_2$D [3.1(0.33), 7.1(0.61), 7.1(0.36) and 9.0(0.57) nmol/; $P<0.0001$] for 0, 12, 24 and 48kIU, respectively. Plasma 1,25$(OH)_2$D concentrations were (geometric mean(95%CI)): 123(69-220), 125(68-234), 145(88-241) and 143(98-210) pmol/L ($P=0.04$). Chromatograms showed a significant increase in the ratio between the peak intensity of respectively 25(OH)D and 24,25$(OH)_2$D and 2 yet unidentified peaks with the same MRMs of these two compounds but with different retention times.
Conclusions
Plasma 25(OH)D_3 $t_{1/2}$ was not influenced by vitamin D supplementation, despite higher plasma 25(OH)D and 24,25$(OH)_2$D concentrations. This suggests that, in contrast to previous assumptions, 25(OH)D_3 $t_{1/2}$ may be closely regulated and mechanisms other than 25- and 24,25-hydroxylation may be involved in the increased clearance of vitamin D at a high oral supply.

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LB12

The role of Creld2 in skeletal development and homeostasis

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Cysteine-rich with EGF like domains 2 (Creld2) has recently been identified as an endoplasmic reticulum (ER) stress inducible gene in the context of skeletal dysplasia caused by mutant protein accumulating in the ER eliciting an unfolded

protein response (UPR). *Creld2* was originally implicated in ER stress following the treatment of Neuro2α cells with thapsigargin. Furthermore, the promoter of *Creld2* contains an ER stress activating transcription factor 6 (ATF6) response element. The function of Creld2 is largely unknown despite various roles being identified in protein folding and trafficking. *Creld2* is expressed in mouse embryonic skeletal tissues and interestingly a novel role has been identified for Creld2 in mesenchymal stem cell (MSC) osteogenic differentiation.
In order to determine the specific role of *Creld2* in bone development and homeostasis, conditional *Creld2* knockout mouse models were generated. Floxed *Creld2* mice were bred onto *Cre* mice to generate cartilage (*Col II-Cre*) or bone (*OC-Cre*) -specific knockout mice.
Recent work has shown that cartilage-specific *Creld2* knockout mice display disproportionate short statue and a disrupted cartilage growth plate with reduced chondrocyte proliferation. Preliminary phenotyping of the bone-specific *Creld2* knockout model has shown that mice develop osteopenia potentially due to an increase in the number of osteoclasts.
The work presented here shows that *Creld2* plays distinct roles in bone and cartilage as cartilage-specific knockout mice are significantly smaller and bone-specific knockout mice have a lower bone density. Future work will focus on underpinning the genetic and signalling changes following the ablation of *Creld2* in order to gain a deeper understanding of its role in bone formation and growth.

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LB13

High-fat diet can accelerate the bone loss process of ovariectomized rats?

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After many years of study, researchers show that obesity can harm the bones. It is not known whether the increased fat mass can accelerate the process of bone loss. The aim of this study was to evaluate whether high-fat diet accelerates the process of bone loss in ovariectomized rats. This study was approved by the Ethics Committee of our Institution (188/2013). 40 female wistars rats with body mass of 60 g were used in this study. They were equally divided into four experimental groups which HFD45: ovariectomized rats fed with high-fat diet, they were killed 45 days after ovariectomy; HFD90: ovariectomized rats fed with high-fat diet, they were killed 90 days after ovariectomy; SD45: ovariectomized rats fed a standard diet, they were killed 45 days after ovariectomy; SD90: ovariectomized rats fed a standard diet, they were killed 90 days after ovariectomy. After the corresponding trial each group the rats were killed by overdose of anesthesia. The tibiae were evaluated when the BMD, Maximal Load. Statistical analysis was performed using SPSS. For BMD, the variable of diet was not influence ($P=0.896$) as well as the time variable ($P=0.986$). However, to Maximum Load the diet variable was not influent ($P=0.146$), but time was influential ($P=0.015$) and the interaction diet*time was influential ($P=0.046$). In conclusion, the high-fat diet does not accelerate the process of reduction in BMD, but accelerates the process of bone fragility in ovariectomized rats.

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LB14

Mechanical properties of rat tibia bone defect after treatment with latex membrane

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In recent years, latex membrane synthesized from natural latex extracted from rubber tree *Hevea brasiliensis*, has been studied as an important biomaterial to assist in tissue regeneration process, since it has ability to induce neovascularization and tissue proliferation, and is a low-cost and easy material handling. The aim of this study was to evaluate mechanical properties of bones after treatment with natural latex membrane in bone defects created in the tibiae of rats. This study was approved by the ethics committee on animal testing of Medical School of Ribeirão Preto/University of São Paulo under the Protocol 61/2015. 15 *Wistar* rats with body mass 300 g were used in this study. Bone defect with 2 mm in diameter was performed on each left tibia of animals. The animals were divided in two groups: C: Animals with no specific treatment ($n=7$) and L: Animals were treated with latex membrane ($n=8$). A latex membrane was placed over the bone defect during surgery in animals of L group. After 4 weeks, the animals were killed and left tibias were removed for mechanical analysis. A Universal Test Machine was used to evaluate the properties of Maximum Load and Stiffness, using the three-point flexion test. The speed of force application was 1 mm/min in the postero-anterior direction. The results showed that there were no statistical differences between the groups for Maximal Load ($P>0.05$) the stiffness was higher in the group treated with latex membrane ($P<0.05$) compared to the group with achieved a greater stiffness ($P<0.05$) compared to the without specific treatment group. The latex membrane may improve the stiffness of the tibia of rats after bone defect.

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LB15

siRNA-mediated Noggin inhibition enhances osteogenesis and mineralization

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Non-unions and critical size defects (CSD) are major orthopedic challenges. The gold standard for CSD treatment, bone grafting, is associated with several morbidities. Growth factors such as bone morphogenetic proteins (BMPs) have been utilized in clinic. Nevertheless, huge doses are required for therapeutic effects. Endogenous BMP antagonists, such as Noggin, could be inhibited using small interfering RNA (siRNA) to increase the bioavailability of BMPs and accelerate bone healing. Lipid-based nanoparticles (LNPs) are the most advanced delivery vehicles. We hypothesize that Noggin siRNA delivery by LNPs enhances osteogenesis and bone healing in CSD. We plan to test our hypothesis both *in vitro* and *in vivo*. We have characterized the physicochemical properties of an LNP and have assessed the ability of the LNP-siRNA complex to transfect cultured osteoblasts. Treatment with 50 nM fluorescently-labeled siRNA resulted in over 90% cell transfection and excellent cell viability, confirmed by FACS and confocal microscopy. Screening of a library of Noggin siRNAs revealed one siRNA with 60% *Noggin* knock-down after 24 h and 70% protein down-regulation after 48 h and a consequent 40% up-regulation in BMP downstream genes, *Smad1* and *5*. We observed a significant increase in both Alp activity and mineralized matrix formation after 10 days of LNP-siRNA treatment. *in vivo* effects of the LNP-Noggin siRNA system will be evaluated in a rat femoral CSD model. Preliminary results from serial *in vivo* imaging demonstrate localized accumulation of fluorescently labeled siRNA at the site of bone defect over time. Treated animals will be sacrificed at different time-points and the bone samples will be assayed by Western Blot, RT-qPCR and immunohistochemistry. The quality and quantity of newly formed bone will be evaluated by X-ray, μ-CT scan, histology, and biomechanical testing.
This study will provide a practical approach to accelerating bone formation via endogenous BMP signal up-regulation and enhancing the potency of minimal safe doses of exogenous BMP. The results from this study will pave the way for the safe clinical management of impaired bone healing by avoiding the administration of toxic doses of exogenous BMP.

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LB16

Proposal for a full-body suspension model for osteopenia induction

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Introduction
Osteopenia and osteoporosis are osteometabolics disorders characterized by decreased bone mineral density (BMD) with deterioration of bone micro-architecture. Since the beginning of the space age, there was a growing interest in trying to reproduce on Earth an environment that could simulate the effects of microgravity to induce bone, a state of osteopenia. The aim of this study was to present a new osteopenia induction model through the full body suspension.
Method
Methods were used eighteen (18) race female Rattus norvegicus Albinus, Wistar variety, with 10 weeks of age and body mass between 200 and 290 grams. The

animals were divided equally ($n=9$) in 2 groups: GS21 – Suspended Group for 21 days; GC21 - Control Group 21 days. The animals in the experimental group were suspended for 21 days through a garment made of rubberized fabric, slightly elastic and trabecular. This garment was arrested by rings on a wooden platform that was embedded in the edges of the cage Beira Mar, keeping the suspended animals. The rats were euthanized and after autopsy and dissection, the analyzes were performed. The bone tissue was analyzed for bone mineral density, strength, bone microarchitecture (through computed microtomography and scanning electron microscopy).
Results
There was a significant reduction in bone mineral density of the humerus and tibia to densitometric analysis; there was a reduction of the maximum strength and relative stiffness of humerus and tibia to mechanical bending test on three points.
Conclusion
The proposed protocol whole body suspension reduced bone mineral density and mechanical strength of the tested bones and was effective for inducing osteopenia.
Key Words: osteopenia; sedentary lifestyle; full body suspension; bone density
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LB17

Efficacy of the therapeutic compliance in the brittle bone disease and in the elderly people frailty

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Objective
The study evaluated the efficacy of the therapeutic compliance in brittle bone disease applied to frail elderly people affected by osteoporosis and in treatment with Denosumab.
Methods
We studied people ≥ 75; 43 women (mean age 83 ± 3) and 14 men (mean age 85 ± 3), all suffering from femoral osteoporosis T-score < -4. In 34 women and 6 men, we discovered 1–2 vertebral fractures. They have been treated with Denosumab 60 mg in a 6-month span of time and Colecalciferolo 50,000 UI every 30 days. The study design included at T0-T24-T48.
Results
At T0 we considered: i) Geriatric Depression State: mild depression 40.5% $P<0.05$, severe depression 53.6% $P<0.05$; ii) Numerical Rating Scale: moderate pain 25.6% $P<0.01$, severe pain 74.4% $P<0.01$; iii) Tinetti balance and gait Scale: mean score 10 (high risk of fall) 83.8% $P<0.5$; mean score 1(non walking) 16.2% $P<0.5$. Finally at T 48 we detected: i) Geriatric Depression State: mild depression 69.3% $P<0.05$, severe depression 24.7% $P<0.05$; ii) Numerical Rating Scale: mild pain 37.3% $P<0.01$, moderate pain 28.1% $P<0.01$, severe pain 9.5% $P<0.01$; iii) Tinetti balance and gait Scale: mean score 21 (low risk of fall) 86.5% $P<0.5$, mean score 10.7 (high risk of fall) 7.6%. At T0 we detected: MMSE mean score 19.5; mean comorbidity index 3; mean severity index of comorbidity 2.23. Between T24 and T48, through X rays of the spine in the dorsal, lumbar and sacral regions, we checked that no new fractures had occurred. At T48 the DEXA Bone Densitometry showed a mean femoral T-score -2.9.
Conclusion
We evaluated the frailty markers (depression, pain, fall) and, after the Denosumab treatment, the efficacy of the therapeutic compliance compared to the changes of the scores and to the quality of life's improvement.
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LB18

Evidence that CD146 positive bone marrow stromal cells in bone metastases have an inhibitory role in regulating tumor growth

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The concept of the vicious cycle of bone metastases emphasizes the cross-talk between osteoclasts, osteoblasts and tumor cells, but the role of bone marrow stromal cells (BMSCs) is not well delineated. BMSCs comprise of a fibroblast-like cell population which adhere to culture flask and have an ability to differentiate into osteoblasts, adipocytes and chondrocytes. They express a panel of cell surface antigens which includes CD105, CD73, CD90, CD106, and CD146. In this study we aimed to further characterize this heterogeneous cell population with a specific focus on the role of CD146 positive cells.
Bone marrow cells from the tibia of C57BL/6 mice were flushed and treated with red blood cell lysing solution to obtain a population of mononuclear cells. Cells were cultured on coverslips for 18–30 days and stained for osteogenic and fibroblastic markers. Staining for CD146 was also performed on tibia harboring bone metastases. Staining was performed on untreated mice or those treated with alendronate.
The majority of cells expressed abundant amounts of α-SMA and COL1A1 while CD146, FAPα and FSP-1 were expressed at low levels only by a sub-population of cells. No Desmin staining was observed. To determine a potential role of CD146 positive cells *in vivo*, a mouse model of breast cancer bone metastases was utilized. The presence of CD146 positive cells was examined by immunohistochemistry. Mice treated with alendronate had reduced tumor size compared to untreated controls. Surprisingly, there was a 60% increase in the presence of CD146 positive cells in the tumor region with alendronate treatment ($P<0.05$).
The data support the premises that BMSCs are inherent osteoprogenitor cells. The increased abundance of CD146 positive sub-population with alendronate treatment indicates that this cell type has a potential role in inhibiting tumor growth. Further evaluation of the role of CD146 in bone metastases is required.
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